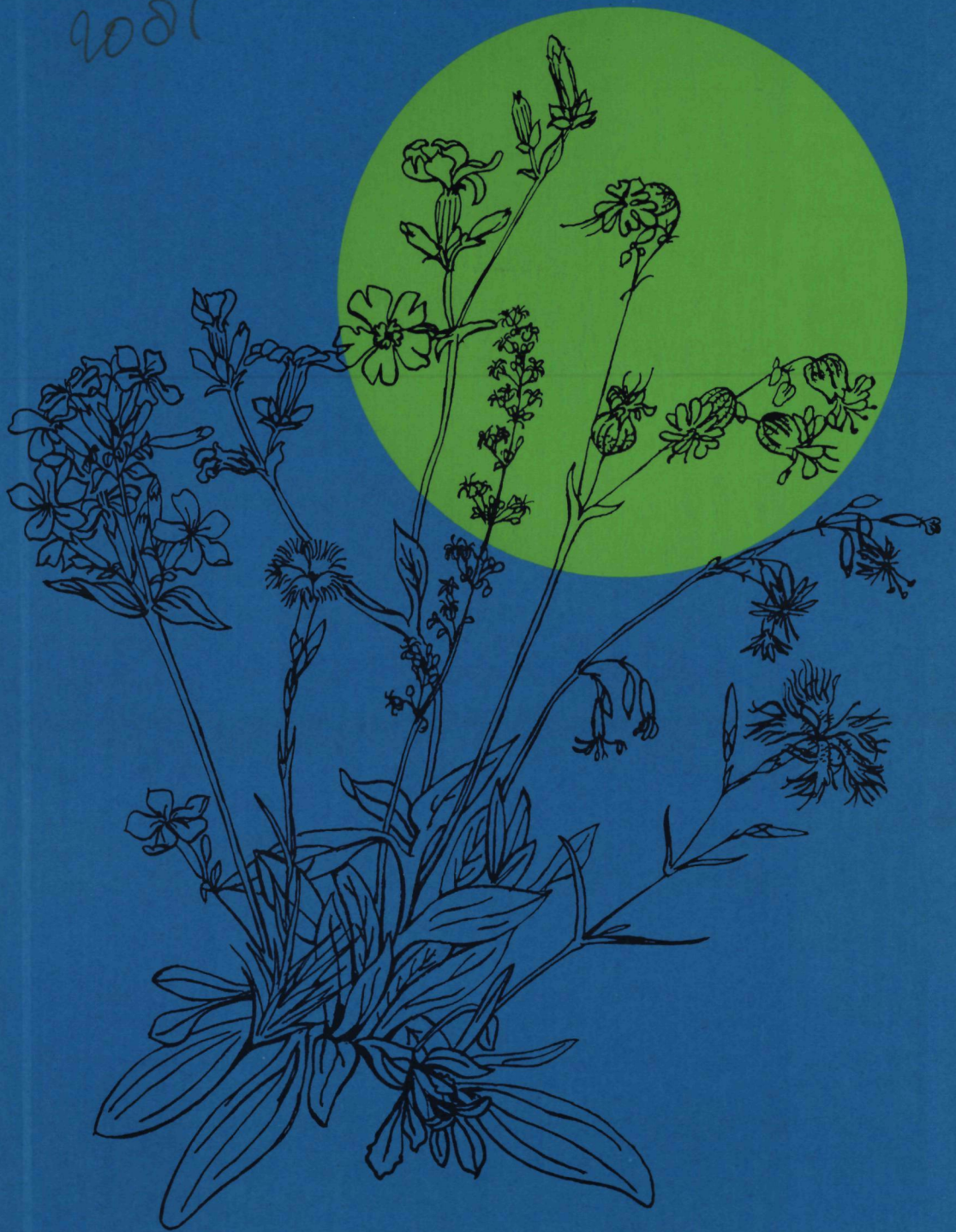
oecologisch onderzoek
BLOEMENGEUR BIJ MANESCHIJN,
met nachtvlinderbloemen

N.B.M. BRANTJES



BLOEMENGEUR BIJ MANESCHIJN,

OECOLOGISCH ONDERZOEK MET NACHTVLINDERBLOEMEN

PROMOTOREN:

PROF. DR. H.F. LINSKENS
PROF. DR. L. VAN DER PIJL

BLOEMENGEUR BIJ MANESCHIJN,

OECOLOGISCH ONDERZOEK MET NACHTVLINDERBLOEMEN

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE
WISKUNDE EN NATUURWETENSCHAPPEN
AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN, OP GEZAG VAN
DE RECTOR MAGNIFICUS, PROF. DR. A.J.H. VENDRIK,
VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN
IN HET OPENBAAR TE VERDEDIGEN
OP DONDERDAG 23 SEPTEMBER 1976
DES NAMIDDAGS TE 2 UUR PRECIES

door

NICOLAAS BERNARDUS MARIA BRANTJES
geboren te Barneveld

Druk: Offsetdrukkerij Faculteit der Wiskunde en Natuurwetenschappen
Nijmegen

Daar het volgens het promotieregelement, artikel 23.1, van de Katholieke Universiteit van Nijmegen niet is toegestaan individuele leden van het wetenschappelijk corps te danken, wil ik mijn dank voor de prettige samenwerking richten aan alle medewerkers van het Botanisch Laboratorium, aan de medewerkers van enkele algemene diensten van de Faculteit Wiskunde en Natuurwetenschappen en aan één medewerkster van het Psychologisch Laboratorium van deze Universiteit.

Ter nagedachtenis aan mijn moeder

Aan mijn vader

INHOUD

1) Brantjes, N.B.M. & Linskens, H.F. (eds.): Pollination and dispersal. Publ. Dept. Botany, Nijmegen: 26-46 (1973).
2) Ent. Exp. & Appl. 20: 1-7 (1976).
3) Proc. Kon. Ned. Akad. Wet., ser. C, 79: 1-12 (1976).
4) Proc. Kon. Ned. Akad. Wet., ser. C, 79: 125-141 (1976).
5) Oecologia 24: (1) (1976).

INLEIDING

Het syndroom van de bloem-bestuiving door nachtvlinders is goed omschreven en geanalyseerd in Faegri & van der Pijl (1971). Het bestuivingsproces zelf is nog weinig onderzocht. In dit proefschrift wordt gepoogd de bestuiving door nachtvlinders experimenteel te benaderen.

Alvorens hun relaties met bloemen te bespreken delen wij de nachtvlinders op basis van hun gedrag bij het bloembezoek in drie groepen in.

1 De pijlstaarten (Sphingidae) kunnen in de lucht stilhangend uit bloemen drinken. Onderzoek is gedaan aan drie soorten: *Deilephila elpenor*, *Manduca sexta* en *Macroglossum stellatarum*, (Brantjes 1973).

2 De uilen (Noctuidae) landen altijd op de bloemen en nemen voor drinken een tamelijk preciese positie op de bloemen in. Onderzocht zijn twee soorten: *Cucullia umbratica* (Brantjes 1976 a) en *Hadena bicruris* (Brantjes 1976, b, c, d).

3 Een derde des nachts actieve groep bestaat uit kleine vlinders. Het zijn meest spanners (Geometridae) en zogenaamde micro's (Microlepidoptera). De vlinders landen op de bloemen en nemen meestal geen vaste posities in bij het drinken. Deze zullen niet verder behandeld worden.

Alle nachtvlinderbloemen hebben een zeer sterke zoete geur, waarvan de functie onvoldoende duidelijk is. Het is onwaarschijnlijk dat de geur louter toevallig geproduceerd wordt.
In analogie met relaties van bloemen met andere bestuivers kan vermoed worden dat de geur de nachtvlinders aanlokt. Daarbij kan de oriëntering met behulp van geur mogelijk het slechte gezichtsvermogen 's nachts van de vlinders compenseren. Slechts enkele waarnemingen kunnen dit bevestigen (Tinbergen 1953). Voor *Autographa gamma* (Noctuidae) toonde Schremmer (1941) het belang van geur bij het bloembezoek, voornamelijk als oriëntatiemiddel, aan. Via experimenten kwam Knoll (1922,1925) daarentegen tot de conclusie dat de geur voor pijlstaarten geen functie heeft bij bloembezoek, en zeer wel gemist kan worden.

Het eerste doel van mijn onderzoek was de mogelijke tegenstrijdigheid tussen de aanwezigheid van de geur en de schijnbare afwezigheid van een functie daarvan voor pijlstaarten op te lossen.

Indien geur een functie heeft bij de oriëntatie blijft de tweede vraag, op welke wijze nachtvlinders zich op de geur oriënteren, bestaan.

De derde vraag is dan of bloemgeur naast oriëntatiemiddel nog andere functies heeft, zoals bijvoorbeeld inductie van specificiteit van de bezoeker, zoals Faegri & van der Pijl (1971) stellen.

Deze drie vragen zullen voor enige pijlstaarten en uilen verduidelijkt worden.

Van één geslacht van de Noctuidae is er een zeer speciale relatie met bloemen bekend. Namelijk, vlinders van het genus *Hadena* bezoeken bloemen van de Caryophyllaceae zowel om er uit te drinken als om er eieren in te leggen. De rupsen eten dan later van de zich ontwikkelende vruchten.

Het leek nu van belang na te gaan wat het karakter is van deze symbiose, in de originele betekenis van De Bary (Schaede 1962). Is de relatie voor plant en dier profijtelijk? Of is de vlinder een predator?

Daarnaast zijn de factoren, speciaal de bloemgeur, die het bloembezoek en het eileggedrag van *Hadena bicruris* beïnvloeden geanalyseerd (Brantjes 1976 b, c, d). Hierbij kreeg speciale aandacht de opmerkelijke capaciteit van de vlinders om stamper- en meeldraad-bloemen van *Melandrium album* te onderscheiden.

In de hierna volgende hoofdstukken zullen de resultaten van de experimentele studie van de bloembestuiving, voor de verschillende - hier voorgenoemde - vlinders afzonderlijk, behandeld worden. Daarna zal in een overzicht de rol van de bloemgeur bij de bestuiving besproken worden, waarbij wordt uitgegaan van het ervoor beschreven experimentele materiaal, aangevuld met enige gegevens uit de literatuur. Tot slot zal de aard van de symbiose van *H. bicruris* met *M. album* besproken worden.

De kwantitatieve studie van de bloembestuiving is voornamelijk in het laboratorium verricht. Dit heeft de voordelen dat het daar mogelijk is de omstandigheden constant te houden. Bovendien kan daar met proefdieren gewerkt worden waarvan de levensgeschiedenis bekend is. Deze voordelen lijken van belang om invloed van de eventuele leerprocessen te onderkennen. Nadeel is echter dat de condities hier kunstmatig zijn waardoor de dieren mogelijk een afwijkend gedrag vertonen. Om te controleren of de conclusies ook geldig zijn voor de veldsituatie zijn intensief waarnemingen gedaan in de Botanische tuin van de Universiteit in Nijmegen en op enige natuurlijke groeiplaatsen van de proefplanten.

REFERENTIES

*) Brantjes, N.B.M., 1973. Sphingophilous flowers, function of their scent. In: Brantjes, N.B.M., Linskens, H.F. (eds.): Pollination and dispersal, Publ. Dept. Botany, Nijmegen: 26-46.

*) ----- 1976 a. Senses involved in the visiting of flowers by *Cucullia umbratica* (Noctuidae, Lepidoptera). Ent. Exp. & Appl., 20: 1-7.

*) ----- 1976 b. Riddles around the pollination of *Melandrium album* (Mill) Garcke (Caryophyllaceae) during the oviposition by *Hadena bicruris* Hufn.(Noctuidae, Lepidoptera), I. Proc. Kon. Ned. Akad. Wet. ser. C 79: 1-12.

*) ----- 1976 c. Riddles around the pollination of *Melandrium album* (Mill) Garcke (Caryophyllaceae) during the oviposition by *Hadena bicruris* Hufn. (Noctuidae, Lepidoptera), II. Proc. Kon. Ned. Akad. Wet. ser. C 79: 125-141.

*) ----- 1976 d. Prevention of superparasitation of *Melandrium* flowers (Caryophyllaceae) by *Hadena* (Lepidoptera). Oecologia: 24 (1)

Faegri, K., Pijl, L. van der, 1971. The principles of pollination ecology. Pergamon Press, Oxford.

Knoll, F. 1922. Lichtsinn und Blütenbesuch des Falters von *Macroglossum stellatarum*. Abh. Zool.- Bot. Ges., Wien 12: 127-378.

----- 1925. Lichtsinn und Blütenbesuch des Falters von *Deilephila livornica*. Z. vergl. Physiol. 2: 329-380.

Schaede, R., 1962. Die pflanzliche Symbiosen. Fischer, Stuttgart.

Schremmer, F., 1941. Sinnesphysiologie und Blumenbesuch des Falters von *Plusia gamma* L. Zool. Jahrb. Syst. 74: 375-435.

Tinbergen, N., 1953. Social behaviour in animals. Methuen & Co. London.

*) = Deze artikelen maken deel uit van dit proefschrift.

SPHINGOPHILOUS FLOWERS, FUNCTION OF THEIR SCENT

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I INTRODUCTION

A very obvious characteristic of the sphingophilous syndrome is the sweet fragrance, mostly emitted with a pronounced nocturnal periodicity. One wonders why the fragrance is so strong, since the Sphingidae have an acute sense of smell. This is, for instance, obvious from their oviposition exclusively on the scent (which is for humans often imperceptible) of specific plants. There exists only general speculations about its function of "attraction" for the moths, (Faegri & van der Pijl, 1971). Baerends (1950) presumes that scent might have a "sign stimulus" function, releasing the feeding pattern of the hawkmoths. However, there exists hardly any evidence for this hypothesis, mainly based on analogy with other Lepidoptera. The function of odour, with respect to flower visits by Sphingidae, seems even to be nullified by the experiments of Knoll (1922, 1925). The aim of the present study is to differentiate between separate functions, viz. the function of (a) releasing, of (b) atracting or (c) means of selection and to determine the importance of each of these aspects.

A general model of behaviour is offered by Tinbergen (1950, 1951, 1955) and discussed by Hinde (1961). The animal possesses different behaviour patterns, each activated by an Innate Releasing Mechanism. We use the term, restricted by Tinbergen (1955) to cases where only one specific sign-stimulus releases only one special pattern. The release of the specific behaviour pattern, or an instinct, includes a series of hierarchically organised steps, which have to be followed successively by the animal to reach a specific result. The concluding activity is used to denominate the whole series. Mostly only a few out of numerous stimuli acting on the animal are effective for each step, and cause the release of the next step.

The feeding behaviour of *Macroglossum stellarum L.* has been studied by Knoll (1922). He described spontaneously occurring changes of mood,"Stimmungsänderungen". One, the feeding mood, is recognizable by the special feeding flight, "Nahrungsflug". However there is no remark on the determining factors. The flight pattern, which I prefer to

call "seeking", is characterized by Knoll as an undulating flight, with sudden drops and large irregular loops through the cage. In my work scent has turned out to be the sign-stimulus for this event, as was hypothesized by Baerends.

The present analysis of the behaviour leading to drinking from flowers and which is called "feeding behaviour", is characterized by its first phase: "seeking" as described by Knoll. However, three reservations have to be made for this behaviour and for the model in general. First it remains to be proven that seeking, used as an indication of the initial phase of the feeding behaviour, is not also the introductory phase for another behaviour pattern, e.g. the reproductive behaviour. Secondly, the specificity of responses to odours can also be effected by selective sensitivity of the sense organs. Thirdly, the innate character of releasing mechanisms is not always absolute: the absence of learning remains to be proven. These three reservations are evaluated with the obtained experimental evidence in the discussion.

Knoll (1922) founds his conclusion that flower scent is of no importance for the diurnal moth *Macroglossum stellatarum* on the fact that a fresh moth does not approach coloured objects in the first day, while flying during three periods, but does so after a confinement of three days. Since flower visiting Sphingidae feed during the first day after pupal emergence, the absence of odour could be the determining factor in the abstination from feeding. Therefore, the conclusion may have to be reserved. The reported spontaneous feeding activity on coloured objects, after three days is the effect of starvation, as will be demonstrated later, and consequently does not support the earlier conclusion. Other experiments with the nocturnal moth *Hyles livornica l.* (Knoll 1925), also leading to the conclusion that scent has no function have a weakness because of the use of starved moths. The possibility of an odorous background from the outside has also not been excluded. Ilse (1928) states that *D. elpenor* approaches coloured objects spontaneously, but uses sprayed perfumes to raise the "feeding mood" of the Lepidoptera in her work, and she gives no information about a possible starved condition of the specimens.

Any orientational function of scent has to be devided into: (a) distance, (b) nearby orientation and (c) final guidance by scent. The latter (c) enabling orientation in the flower, occurs always in combination with visibility. The first (a) and second (b) activities can be distinguished by two criteria. Firstly, the existing differen-

ces in odour concentration, viz. being weak in the first case, and more pronounced in the second case. Secondly by the existence of additional stimuli as will be the case with the second function. Distance orientation, based on the second criterion, can be simulated in an experimental cage by a hidden source of scent. Reservations have to be made here for the generally high concentration and for the disturbance of a concentration gradient from the source by air turbulences caused by the flying animal. Distance orientation on scent, in which I include the scent-released anemotactic orientation of *Bombyx mori* (Schwinck 1954, 1955) has only one alternative, e.g. random flight. Nearby orientation to odour can be compensated or improved by visual orientation, which allows faster and therefore more economical approach. Tinbergen (1956) observed attraction of *Hyloicus pinastri L.* by a hidden honey suckle flower in a forest. My experiments in this respect with four moths failed, probably due to inappropriate conditions. According to Morgan (1928), amylsalicylate and several related chemicals, will attract *Manduca sexta* to artificial flowers. This observation was made under experimental as well as field conditions. However, the possibility of optical orientation, released by scent, cannot be excluded.

Odour may also cause specificity in visits to flowers. The insect is able to distinguish plants by their scent, and does so e.g. for oviposition. The importance of the distinguishing of odour by sphingidae while visiting flowers has been one of the subjects of this study. The emission of special odours by the plant might cause selection of visitors, either by repelling or by attracting. Flowers have few structural means for the exclusion of sphingidae, since the insects long thin proboscis and the ability to hover guarantee access to all kinds of nectar supplies. In order to save their nectar for more effective pollinators, flowers, not pollinated by visiting hawkmoths, have to deter them from visits by emitting specific repellents or by avoiding the production of odours attractive to sphingidae, or by offering nectar in insufficient quantities for moths.

Sphingophilous flowers may be adapted to select between different sphingid species by differences of tube or spur lenght. In several Oenothera species, however, lengthening of the hypantium does not exclude the short tongued sphingidae (Gregory 1964). Perhaps the emission of specific scents by the plant will induce visitors to constancy. Whether this works, depends on the existence of either innate scent specific preferences or on the ability to show conditioning phenomena to such scents. *Hyles lineata livornica* Esper is neither

selective nor constant as is revealed by the analysis of pollen grains on the proboscis by Kislev (1972), and is also the case in other sphingidae in Israel (Eisikowitch 1972). *Hyles livornica* L. and *Macroglossum stellatarum* L. visit all kinds of coloured objects as long as they offer nectar, irrespective of odours (Knoll 1922, 1925).

Constancy based on a learned or on an innate colour preference is possible (Knoll 1925) and may even explain the following unexplained field observation of Gregory (1964): *Pholus agemon Drury, Xylophanes tersa* and *Hyles lineata Fabr.* are constant in a mixed population of differently coloured flower species, but not in a mixed population of several yellow *Oenothera* species.

I used *Deilephila elpenor* and *Manduca sexta* for my experiments. *D. elpenor* is a regular visitor to *Lonicera periclimenum* but also of other flower species. Flower visits of *M. sexta* have been reported by Knuth (1905), Gilmore (1938), Madden (1945) and Gregory (1964). From the many plants visited, I have selected *Petunia hybrida H19; Ka 30*. Only one experiment was made with *Macroglossum stellatarum.*

M. sexta frequents *Petunia* flowers in nature, and since the the stigma and stamens are situated in the corolla tube, only contact with the tongue is possible. According to Gregory (1964) pollination by means of contacting the reproductive organs of *Oenothera* flowers with only the proboscis of a sphingid is low. Since he related it to the stickyness of *Oenothera* Pollen, pollination will even be worse with the loose pollen of *Petunia*. Therefore, it was regarded worthwile to find out whether *Petunia* is indeed pollinated by *M. sexta*, and whether some information could be obtained about the long range transport of pollen by this hawkmoth.

A real function of the flower scent exists for various other flower visiting Lepidoptera. For many Rhopalocera scent can act as sign stimulus for the feeding instinct, as discovered by Knoll (1922) and Ilse (1928). In the noctuid *Autographa gamma L.* scent also releases the feeding behaviour, and scent can act as a means of orientation to and distinguishing of the flowers. Schremmer (1941) also discovered a learned constancy based on scent. However, for Noctuids also innate odour preferences occur in the feeding behaviour, as I discovered for *Hadena bicruris L.* which in addition to drinking also oviposits in the flower of *Melandrium album* and close relatives.

II MATERIAL AND METHODS

All experiments have been carried out in a square cage according to the description by Knoll (1922), except somewhat larger: 60x60x60 cm. The walls of white cotton were stained with black dots to enhance the visibility. A movable glass screen at the front allowed observations. A transformer regulated the light intensity of an incandescent lamp, which projected a light spot of 10 cm diameter on the center of the right wall. By wetting the walls with distilled water before each experiment, the relative humidity was raised to more than 95% for two hours. To prevent unnoticed contaminations by odour, the following precautions were taken: perfumed human beings were excluded from the experimental room, chemicals and materials were stored seperately and flowers were kept outdoors until the moment of introduction into the cage.

Artificial flowers were folded from coloured paper in the model "Trichterblume" of Knoll (1925, p. 239) with a diameter of 2 cm and dipped into melted paraffin, fig. 1. Another model used was the"pipet flower", made of a 1cm square of white cotton, with a punched hole in the centre, kitted on the brim of a pipet (diameter 5 mm). The constriction in the pipet, 15 mm underneath the cotton was closed with plasticine. When desired, a sugar solution could be deposited in it.

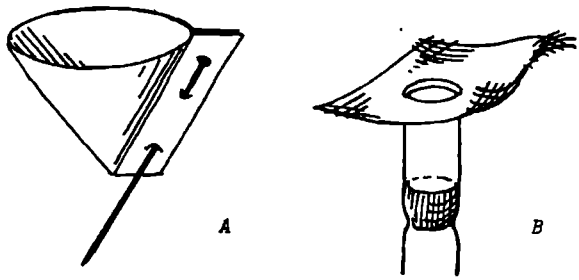

Figure 1, *Artificial flowers, A= Trichterblume, B= Pipet flower.*

For the experiments with *D. elpenor* a scent-source was made from ten flowers of *L. periclimenum* hidden in a cardboard tube (diameter 4 cm, length 10 cm) at both ends closed with black netting, which was placed vertically in the middle of the cage.

*E*lectro *A*ntenno *G*rams were made in the "Laboratorium voor Physiologie der Dieren", in Wageningen of freshly cut antennae. The minimum detectable value was 0,1 mV over the whole antennae. Each test was repeated three times. The odour was introduced into a continuous airflow over the antennae. Blanks: distilled water and Apiezon L grease gave no response. When no fresh flowers were available, the "enfleurage" from them was used. Thus is the fragrance of the flower dissolved in grease (Apiezon L).

Most flowers were grown in the botanical garden of the University of Nijmegen: *Datura stramonium, Oenothera biennis, Epilobium angustifolium, Phlox paniculate, Melandrium album, Lilium longiflorum, Lilium "sentinel", Hyacinthus orientalis, Petunia inflata.*
Grown in the glasshouse were *Petunia hybrida* clone H19; Ka30. *Lonicera periclimenum* was gathered in a forest in the neighborhood of Nijmegen.

Chemicals were generously provided by the companies "Chemische Fabriek Naarden" and "International Flavour and Fragrances".

All animals were kept in a normal day light rhythm. Experiments were only carried out during the night. Experiments in scentless atmosphere were always performed before experiments using scent. When several insects were to be used within one night, they were placed in an odourfree room under a lamp, and used one after another in the experimental room. Because of shortage, individual moths had to be used in several experiments. All conditions were protocollized individually to exclude unnoticed interactions. Moths are called "experienced" as soon as they have been fed or have made a rewarding visit to scentless artificial llowers or a visit to a flower. The behaviour of the moths was studied by making flash photographs. After a flash the moth drops on the floor. Therefore, after the photograph was made the experiment was always ended.

The number of observations for each statement varied greatly. Often the number was small and no calculations of significance were made. A repetition of a complex behaviour by change only seems to me to have a very low probability. Therefore, I have considered one repetition of a complex behaviour as a guarantee for its significance. The frequency of observations, which led to a particular conclusion, is presented between brackets to most statements.

Two *Macroglossum stellatarum L.* were caught in the Gargano in Italy and transported to Nijmegen in a dark container of one liter. Each second day they were fed till satisfaction with sugar solution. The first experiment was the seventh day after capture. Several moths of *D. elpenor* emerged in the laboratory and therefore were unexperienced. Three insects were trapped with light in the field; these were absolutely undamaged and lived for more than twenty days under experimental conditions, and therefore they might be considered rather fresh too. Our *Manduca sexta John.* has been derived from a culture grown on an artificial diet at the Wageningen laboratory. Consequently these insects were of known age and experience.

III OBSERVATIONS

Behaviour, Deilephila elpenor: In the daytime, the moths sit against the wall with their heads upwards, or often hang on one foreleg. In the resting state the wings are folded rooflike over the abdomen and the antennae lie backwards. The body remains pressed against the wall. Lowering of the light intensity causes a change to an activated condition: the body is lifted some millimeters, within minutes the antennae move forewards until they form an angle of 60^{o} to 90^{o} with the body axis (n=9). Subsequently a sharp decrease in the light intensity initiates the silent warming up, visible by the shivering of the raised wings, and followed by flight, (n=30 out of 35 times). Before taking off, the proboscis is partly uncoiled for a short moment, and the antennae are rubbed along the spine on the foreleg. This moth can take off from a flat surface, the first flight is always directed towards the light. Hovering in the cage is very good, with only occasionaly a sudden change to fast level flight and a subsequent crash against the wall. In the top of the cage the moth circles slowly and often bumps against lighter spots, as if trying to escape. Lowering the light intensity below 1 lux causes an immediate landing on the floor. A slow increase to 20 lux has no observable effect during flight, and facilitates observation. Need and tolerance of light is significantly higher than that of *M. sexta*, which is next to be discussed. This explains observations on the flight of *D. elpenor* at sunset, whereas Gregory (1964) observed flight of *M. sexta* during the night only.

Behaviour, Manduca sexta: *M. sexta* always sits in the same way, in either the resting or active state as *D. elpenor*. In the cage, flying starts after sunset, but it is prevented by illumination. The here audible warming up starts at an undetermined moment within an hour after lowering of the light intensity to 1 till 7 lux. Disturbing the moth causes an immediate warming up. Sometimes, a fast lowering of the light intensity appears to be sufficient. Take off from a smooth surface is unsuccessfull. The moth flutters up the wall before free flight. While hovering they circle slowly, humming in the top of the cage, avoiding objects. Often a sudden change to a fast straight flight occurs, ending in a crash against the cotton wall.

Activation by scent, Deilephila elpenor: Introduction of the scent of *Lonicera periclimenum* into the cage, containing a moth in the active state, causes warming up within minutes, 6 out of 8 times. Carrying out the same experiment without this scent never causes warming up. The duration of the first and second flight on one evening is prolonged by odour. The average flight duration is for scentlessness 4.0 min. (n=35), with scent 10 min. (n=6). The actual flower visits lasted much longer.

Activation by scent, Manduca sexta: Some scents (Table 2 partI) elicit within 30 seconds a warming up of active state insects only in 4 out of 25 tests, with a duration of approximately 5 min. Odour causes lenghtening of duration of flight from a mean of 2.3 min. (n=21) to 8.5 min. (n=35).

Change of behaviour by scent, Deilephila elpenor: In a scented atmosphere the flight pattern looks like the seeking as is described for *Macroglossum stellatarum* by Knoll. The flying moths change flight from normal "aimless" hovering to seeking, within seconds after introduction of the scent. After removal of the scent source, seeking stops after about 3 min. (n=4), followed by landing and active state sitting. A satisfied moth lands and sits in a resting state. Seeking which always preceeds flower visits, was never observed without presence of the scent of *L. periclimenum* (n=10), mean observation time: 50 min. . All other scents tested turned out to be ineffective, (Table 2). However, moths which were not fed that night sometimes drank on artificial flowers in scentless atmosphere at the end of the night. A moth satisfied

two hours earlier starts seeking and flower visiting, as soon as scent is introduced.

Change of behaviour by scent, Manduca sexta: As observed for *D. elpenor* the flight pattern of *M. sexta* is changed to seeking a few seconds after introduction of the proper odour, (Table 2). For seven nights the moths with cut-off antennae as well as intact insects have flown simultaneously. The first group made just one flower visit, while the intact animals visited flowers every night. Starving insects sometimes drink on artificial flowers in scentless atmosphere at the end of the night. After one successfull visit to a coloured object, the moth continues to visit the objects, also after elimination of the source of scent. After the scent has induced a particular feeding behaviour, this is maintained for a long time irrespective of the presence or absence of odour.

Change of behaviour by scent, Macroglossum stellatarum: The behaviour of the moth in the cage is described by Knoll. The moths were kept for one day in the cage in which four artificial flowers (red, blue, yellowand white) were placed. During the first day the flowers were not visited. In the morning of the next day about five yellow flowers of *Spartium junceum* were added. Within 10 seconds the two hawkmoths made approaches with stretched proboscis to these nectarless flowers. After this event the artificial flowers were also visited. It is concluded from this experiment that the scent of *Spartium* was successfull in releasing the feeding behaviour.

Change of behaviour by scent: From this we conclude that scent can release the feeding drive in all three species of hawkmoths, both during active sitting and during "random flight" in the cage. The feeding behaviour is characterized by the start of seeking. A moth activated to the feeding drive is also motivated to a longer lasting flight in the cage. The drive continues for *D. elpenor* about 3 min. after stop of the sign stimulus, unless the behaviour enters into the following stage after perceiving of flowers. Satisfaction can raise the threshold value of the sign stimulus to a high level. After one hour the feeding drive can be activated again. As will be shown, conditioning of the two hawkmoths to colours only is impossible. The odorous signal has to acti-

vate each time the feeding urge first. In starving moths the feeding behaviour can be released "spontaneously".

Orientation, Deilephila elpenor: Flowers of *Lonicera periclimenum* are put in a cardboard box with only one opening. The opening is in the bottom and shaded from the illumination. The box is placed in the cage at about 30 cm from the floor. After some seeking loops, the moth approaches the hole, hovering over the ground alternatingly swinging to both sides. The moth rises and lands on the brim of the opening. At this moment the proboscis is stretched, as can be seen on flash photographs. Both an experienced and an inexperienced moth approach the source of the scent in this way.

White and coloured flowers, different flowers between glass plates and *L. periclimenum* flowers in an airtight glass jar are never approached in a scentless cage (n=6). With scent atmosphere these objects get only 2 approaches in 9 experiments, although the moth is always seeking. Uncovered flowers of *L. periclimenum* put in the cage are visited within 10 seconds (n=20, with only one exception). The first approach is a slow swinging rise from beneath the flowers. However, the first orientation of the moth is not always carried out with its back turned to the illumination of the cage, as is generally the case with subsequent visits. Consequently, this first near approach may also be guided by scent. The subsequent visits are carried out faster from the same height as the flower or from above. After some visits to flowers with scent, also scentless objects are approached by the moth (n=5). Optical orientation replaces olfactoric after some training. However, while visiting flowers, also invisible scent sources are approached and touched with an extended proboscis (n=5).

When given four scentless pipet flowers with sugar solution alternated with four empty flowers of *L. periclimenum*, it was observed that the scentless flowers are visited with a higher frequency only after 10 visits to both types of flowers. The moth, trained one hour before in this way on scentless flowers, and given only scentless flowers and an invisable scent source, starts with a visit to the scent. I conclude from this that the moth can obtain a preference for a particular visual character, but this preference is not lasting. The innate preference for a special odour replaces it automatically.

To control the relative attractiveness of only visable objects I used the method of Knoll, viz. the staining of the traces made by the proboscis on a glass plate. White flowers, white and blue pieces of paper and squares of cotton, used for pipet flowers, are attractive. Flowers of *Melandrium album* turned out to be more attractive than those of *L. Periclimenum* (n=5). These flowers, between glass plates, are visited on both the illuminated side and the back, The traces are found mainly on the largest area of the flowers, just as Knoll observed with *D. livornica*. The squares of cotton were also tried more often than the flowers of *L. periclimenum*. Red *Phlox paniculata* and yellow *Oenothera biennis* receive few approaches of the moth. No further experiments about colour preference or about learning have been performed.

Orientation, Manduca sexta: In a scentless cage, artificial flowers as well as purple and rosa *Petunia hybrida* flowers in airtight glass jars are avoided, therefore they must have been observed by the moth. As soon as scent enters the cage, the seeking flight is directed to these coloured objects. Hovering in front or above, the moth extends the proboscis (21 times out of 23 experiments).

M. sexta starts the first flower visit to a *Petunia* flower by approaching it from beneath (n=3). From an approximate distance of 20 cm the moth rises until it is in front of the flower and subsequently unrolls the proboscis. This suggests orientation by scent. The moth starts seeking after introduction of a paper of 1 cm^2 drenched with odour, however never approaches it. Perhaps this scent concentration is too high and saturates the smell organs. In that case scent orientation would be impossible since differences in concentration are imperceptible. Scented artificial flowers, without sugar solution, are at first frequented more often and more intensely than scentless ones. After fifteen visits there are no differences anymore.

We conclude from these results that for *M. sexta* vision is sufficient to find the flowers for the first time. After some experiences it becomes the only means of locating the flowers. Whether scent alone can attract the moth is not sure yet. In any case, scent promoted the locating of the flowers by the moths at the first visits. And therefore odour is of help in attraction and distinguishing of visually identical flowers.

Learning: Moths, trained by feeding in a scented atmosphere on artificial flowers the day before, approach the flowers only after scent

emission, (Table 1). There is no durable association: food-coloured objects formed, working on this phase of the behaviour pattern. Scent has to release the feeding behaviour repeatedly. No evidence is obtained about the possibility that in a following stage, e.g. while hovering in front of the flower, preferences for special scents are developed under influence of experience. The approach of flowers is susceptible to a learned colour preference, even influenced by experience of the day before, as has been discovered by the Knoll.

	scentless		scent atmosphere	
	+	-	+	-
Manduca sexta, trained moths	0	6	8	1
untrained moths	0	4	13	1
Deilephila elpenor, trained moths	1	7	8	1

Table 1 *Dependence of visits to artificial flowers on scent and on training the day before. Number of insects: + = visiting flowers, - = not visiting flowers.*

Effectiveness of scent: The effect of several flower scents and chemicals in the two species is different, (Table 2,part 1). The odour of *L. periclimenum* was the only activating and attracting scent for *D. elpenor*. Several odours elecit seeking by *M. sexta*, including compounds which are ineffective for *D. elpenor*. Therefore we may conclude that there exists a specific reaction to scents.

From EAG measurements it is clear that the moths are able to smell all fragrances tested, (Table 2, partII). Consequently, specificity is not due to selectivity of the sense organs. Details of the EAG experiments will be communicated elsewhere. The sensitivity to several compounds is different for each species of the moths. However, there appears to be no correlation between sensitivity and the reaction to a particular scent.

An analysis of the chemical composition of these flower scents will have to be made in order to decide whether a particular compound or a mixture is necessary for effectiveness.

Fragrance of	Part I Behaviour reaction		Part II EAG	
	M. sexta	D. elpenor	M. sexta	D. elpenor
Petunia hybrida	S	-	+	+
Nicotiana alata		-	+	+
Datura stramonium	-			
Lonicera periclimenum		ASO	+	+
Lilium longiflorum	+/-	-	+	+
Sanseveria rotundifolia		+/-		
Melandrium album		-	+	+
Phlox paniculata	-	-	+	+
Gardenia jasminoides		-		
Eugenol	-	-	+	+
Geraniol		-	+	+
Geranyl-acetate	-	-	+	+
Limoneen		-	+	+
Linalool		-		
Vanilline		-		
Methyl-benzoate		-		
Benzyl-benzoate		-	+	+
Isoamyl-benzoate	AS	-	+	+
Methyl-salicylate	-	-		
Benzyl-salicylate	S		+	+
Isoamyl-salicylate		-	+	+
n-amyl-salicylate	AS	-	+	+

Table 2 *Response of Manduca sexta and Deilephila elpenor to odour.*
A = activation of active state insect, S = seeking,
O = orientation to the source of scent, - = no effect obser
+ = positive response in EAG, +/- = sometimes seeking.

Deilephila elpenor visiting flowers of Lonicera periclimenum: The approach to the flower is made with the legs against the body. The antennae remain in a fixed position, directed forewards at an angle of 120° with the body axis and lifted some 45° above the frontal plane. In front of the flower the proboscis is unrolled and the tip repeatedly pressed on the flower, by bending and straightening. As soon as the proboscis slips into the flower tube, the moth hovers towards the flower and stretches its legs, similar to the behaviour of the short tongued *Celerio lineata* (Gregory 1964). While drinking, the moth keeps holding onto the stamen, pistil and lower lip of the flower. The moth rests with its weight partially on the flower, but remains hovering. When touched by the flowerparts, the antennae are moved backwards. After the nectar is finished the head is forced several times against the flower, by a pumping movement of the whole body in order to penetrate with the proboscis deeper into the flower. After hovering backwards the proboscis is rolled in. Within seconds the next flower is approached. The flowers of *L. periclimenum* are situated on terminal heads, arranged in horizontal circles. All flowers are visited sequently by circling of the moth with no preferential direction. After some repeated visits to the same flowers the next head is approached.

Pollination of L. periclimenum by D. elpenor: The moth hovers at some distance from a flower, which is filled to the brim with a sugar solution, and penetrates slowly when the quantity decreases. Consequently, the proboscis is not plunged deep into the liquid as Knoll (1922) states, and will not become wetted and sticky from the nectar. From other observations it can be concluded that the taste organs on the outside of the proboscis are restricted to the outer 3 mm of the tip.

By gripping, the anthers and pistils, the legs, body and head become smeared with pollen. At the approach of the flower the extending anthers and pistil touch the antennae which become even more loaded with pollen than the other parts of the body. Actual pollination was not checked, one visit will however undoubtedly transfer sufficient pollen grains. The poor seed production, which has been observed in *L. periclimenum* (frequented by hawkmoths) in natural habitas around Nijmegen, may have been due to the strong vegetative multiplication by shoots in those plants. This results in vast areas with the same clone, and consequently a high frequency of geitonogamie.

Stimulus	Phase	Reaction
High light intensity, or satisfaction, absence of disturbances	1 ← 21	Resting position
Lowering light intensity, only after 1 day rest or in combination with disturbing	2 ← 22	Active position
Fast lowering of the light intensity or introduction of an effective scent	3	Warming up
Reach of threshold thorax temp.	4	Start flight
Appropriate scent	5	Release feeding instinct, seeking
At first, scent gradient	7 ← 17,19	Approach of the source
After training, visable objects	8	Approach of the object
Distance perception	9	Hovering in front of
Smell as well as vision	10	Unrolling of the proboscis
Vision of the largest area	11	Directing tongue on the flower
Tactile or taste?	12	Piercing the tongue in the opening
Flower parts within reach	13	Gripping the flower
Taste of sugar solution	14	Drinking in a fixed position
End of taste or sucking up air?	15	Pumping against the flower
About a second without reward	16 → 21	Extraction of the proboscis
Not satisfied		
A flower to the side	17 ← 8	Approach of the next flower
Some flowers without reward	18 → 20	Leaving of the flower head
Smell or vision of another flower head	19 → 8	Approach of another flower head
Many unrewarded visits, disappearance of the scent	20	Leaving of the site or landing
Satisfaction	21 → 1	Resting position
At any moment, to low or to high light intensity	22 → 2	Landing and active state sitting

Figure 2 *Scheme of the behaviour of Deilephila elpenor on flowers of Lonicera periclimenum.*

Manduca sexta visiting flowers of Petunia hybrida: The approach is always with the back turned to the illumination source. From a distance of approximately 20 cm the moth hovers slowly towards the flower, while the antennae remain in a fixed position. At a distance of 10 cm the proboscis is unrolled, and relative to the body it is kept in a fixed position. The tongue is repeatedly dipped onto the flower by undulating flight in front of, or above the flower. As soon as the tip enters the corolla tube, the moth lowers itself and the proboscis slips down in the tube, while bending occurs only passively. After some time of hovering stationary, the moth pumps up and down some 10 mm as if to pick up the remains of the nectar.

The moth leaves the flower by a fast flight. Often the proboscis remains stretched for some time afterwards. During visits to *Petunia* flowers or to artificial flowers the legs are kept against the body. By cutting off the corolla and placing it vertically in a glass pipet, a long tube *Petunia* flower was simulated. At the insertion of the proboscis the legs grip the brim of the flower as soon as it comes within reach. The moth remains novering without leaning on the flower. Only the part of the tongue before the bending, about 60 mm, is inserted in the corolla tube.

Pollination of P. hybrida by M. sexta: To inspect the effectiveness of pollination, the pollen grains on the stigma of emasculated flowers were counted after each visit. After several visits to intact flowers the mean number of grains is 252 (n=3) per stigma. In spite of the problems discussed before, *M. sexta* turns out to be an effective pollinator of *Petunia*. When a fresh moth starts with only one visit to a flower with pollen, the mean number of pollen grains deposited on the next visited flower is 59 (n=3). Which means that a stock may have to be formed on the proboscis. At the next three visits to emasculated flowers, the mean yield decreases sharply: 27, 2, 6 pollen grains per stigma (n=3). Thus the stock of pollen falls fast, which most likely can be attributed to the low stickyness of the pollen.

As a consequense pollination within a particular population of *Petunia* may be efficient. However, pollen transfer to another population may be low, because only few grains are transported each journey, and according to Gregory *M. sexta* only occasionally leaves or enters a particular colony of flowers.

IV DISCUSSION

Scent as a releaser of the feeding behaviour: Although seeking is always followed by flower visits when flowers are available, the question remains whether seeking belongs to the feeding pattern exclusively. This question can be transformed to the question whether the determination of the feeding patterns occurs at the perception of the scent or after the seeking, when the flowers are perceived. Because *D. elpenor* also unrolls the proboscis at the hidden flowers of *L. periclimenum*, we conclude that this scent not only releases seeking but also determines the feeding behaviour. Whether other scents release a reproductive behaviour and whether this also starts with seeking remains unsolved. Consequently the use of seeking as indication of release of the feeding behaviour is legal.

The specificity in response to odour might be effected by a restriction of the sensitivity of the sense organs. However, the EAG shows a sensitivity to all scents tested. The selective response is therefore due to a selection in the central nervous system. Only specific odours can act as sign stimulus.

Learning turns out to be of no importance for the release of the feeding behaviour in this situation. Therefore the innate character of the releasing mechanism is concluded.

From all these arguments we can conclude that specific odours can act as sign stimuli to the Innate Releasing Mechanism of the feeding behaviour. This function seems to be general for the flower visiting hawkmoths. The ability of releasing the feeding behaviour may be of great value to the Sphingidae, because it may prevent fruitless search at places where there are no flowers.

Constancy based on scent: Because several compounds effective for *M. sexta* are inadequate for *D. elpenor*, we conclude to a specificity of the activation substances. The number of flower scents effective for *D. elpenor* is small, viz. only one of the tested flower scents. Whether each Sphingid reacts to the scent of only a limited number of flower species will be studied soon. If so, the plants possess a special means of visitor selection. The moth species not

reacting to the flower scent will pass, unless they are motivated otherwise, e.g. in mixed populations. However it remains to be verified if this selection actually occurs. Although visits of *M. sexta* are recorded to flowers of *Datura stramonium*, their scent is indifferent to the moth. Is that scent directed to other Sphingid species? Visits of *D. elpenor* to *Nicotiana, Phlox* and several other species have also been observed, while their scent turned out to be ineffective for the moth.

Scent as a means for orientation: Besides direct orientation to a scent source, there are several alternatives. Firstly, the mechanisms of anemotaxis released by an appropriate scent, as observed by Schwinck (1955) for *Bombyx mori*. Secondly, the fast flying hawkmoths are allowed to cross vast areas and are provoked to seeking as soon as they enter a cloud of perfume. The observations with *Hyles lineata (Fabr.)* at *Mirabilis longiflora* by Baker (1961) and Cruden (1970) might be explained in this way. This ability may compensate the absence of accurately directed distance attraction.

Because *D. elpenor* is able to find a hidden flower by smell, distance attraction by scent is also possible for Sphingidae as is also suggested by the observation with *Hyloicus pinastri L.* by Tinbergen (1958). The absence of this ability in other species has not been proven yet. The attraction of *M. sexta* to scented artificial flowers, as observed by Morgan (1928), does not exclude the possibility of optical orientation, activated by the scent signal. Anyhow, the relative importance of smell and vision in attraction varies for different Sphingidae.

For near orientation, *D. elpenor* is fully dependent on smell at the first flower visits, whereas for *M. sexta, Hyles livornica L.* and *Macroglossum stellatarum* vision is sufficient or is the dominating factor. After several visits optical orientation always prevails. The visual orientation to white objects has been observed by Kugler (1971) with *Agrius convolvuli L.* while flower visits were going on, which means that no conclusion can be drawn whether initial orientation of *Agrius convolvuli L.* is optic or olfactorial. According to Clements and Long (1922) *Hyles lineata (Fabr.), Hyloicus separatus* and *Manduca quinquemaculata Haw.* do not approach invisible fragrant flowers of *Pachylophus*. However, this observation has been made without exclusion of the visable alternatives for the moth. For only optically orientating insects the plant has no need to restrict scent emission to the flower, as long as

there exists a correlation between emission and flowering season.

Selection of flowers: On this low level in the visiting behaviour pattern also selection based on odour can take place. While hovering in front of the flowers, *M. sexta* is able to distinguish flowers according to smell only, and *D. elpenor* favours *L. periclimenum* flowers to the optically more attractive pipet flowers. The question whether this applies to other Sphingidae, and whether it occurs in the field, remains to be solved. Conditioning to a special odour on this level of the behaviour might be possible.

Visual orientation will always suffice for final guidance into the flower. According to Knoll (1925), *H. livornica* directs the proboscis to the largest surface. After this, the structure of the flower guides the tongue mechanically to the nectar. Guiding by smell may be absent.

Feeding behaviour of Deilephila elpenor: A scheme has been designed of this behaviour pattern, fig. 2, which includes only the external factors and some probable internal factors. This scheme seems to be valid for all flower visiting Sphingidae. Only the scent quality, the threshold values of the stimuli and the relative importance of alternative stimuli may differ for different species.

V REFERENCES

Baerends, G.P., 1950. Specializations in organs and movements with a releasing function. In: Physiological mechanisms in animal behaviour. Symp. Soc. Exp. Biol. 4 : 337-361

Baker, H.G., 1961. The adaptation of flowering plants to nocturnal and crepuscular pollinators. Quarterly Review of Biology 36 : 64-73

Clements, F. E., Long, F.L., 1923. Experimental pollination , an outline of the ecology of flowers and insects. Carnegie Institution of Washington.

Cruden, R.W., 1970. Hawkmoth pollination of *Mirabilis* (Nyctaginaceae). Bull. Torrey Bot. Club 97 : 89-91

Eisikowitch, D., Galil, J., 1971. Effect of wind on the pollination of *Pancratium maritimum* L. (Amaryllidaceae) by Hawkmoths (Lepidoptera/ Sphingidae). J. Anim. Ecol. 40 :673-678

Faegri, K., Pijl, L. van der, 1971. The principles of pollination ecology. Pergamon, Oxford.

Gilmore, J.U., 1938. Observations on hornworms attacking tobacco in Tennisee and Kentucky. J. Econ. Entomol. 31 : 706-711

Gregory, D.P., 1963., 1964. Hawkmoth pollination in the genus *Oenothera*. Aliso 5 : 357-384, 385-419

Harris, B.J., Baker, H.G., 1958. Pollination of *Kigelia africana* Benth.. J. West Afr. Sci. Ass. 4: 25-40

Hinde, R.A., 1966. Animal behaviour. McGraw-Hill Book Company, New York.

Ilse, D., 1928. Über den Farbensinn der Tagfalter. Z. Vergl. Physiol. 8 :658-692

Kislev, M.E., Kraviz, Z., Lorch, J., 1972. A study of hawkmoth pollination by a palynological analysis of the proboscis. Israel J. Bot. 21 : 57-75

Knoll, F., 1922. Insecten und Blumen. III Lichtsinn und Blumenbesuch des Falters von *Macroglossum stellatarum*. Abh. Zool.-Bot. Ges., Wien 12 : 123-378

———, 1925. Lichtsinn und Blütenbesuch des Falters von *Deilephila livornica*. Z. Vergl. Physiol. 2 : 329-380

Knuth, P., 1905. Handbuch der Blütenbiologie. III. Wilhelm Engelmann Verl., Leipzig.

Kugler, H. 1971. Zur Bestaubung grossblumiger *Datura*-arten. Flora 160 : 511-517

Madden, A.H., Chamberlin, F. S., 1945. Biology of the tobacco hornworm in the southern cigar-tobacco district. U.S. Dept. Agr. Tech. Bull. 896

Morgan, A.G., Lyon, S.C., 1928. Notes on amyl-salicate as an attractant to the tobacco hornworm moth. J. Econ. Entomol. 21 : 189-191

Schremmer, F., 1941. Sinnesphysiologie und Blumenbesuch des Falters von *Plusia gamma* L.. Zool. Jahrb. Syst. 74 : 375-435

Schwinck, I. 1954,1955. Experimentelle Untersuchungen über Geruchssinn und Strömungswahrnehmung in der Oriëntierung bei Nachtschmetterlingen. Z. Vergl. Physiol. 37 : 19-56, 439-458

Tinbergen, N., 1950. The hierarchical organization of nervous mechanisms underlying instinctive behaviour. In: Physiological mechanisms in animal behaviour. Symp. Exp. Biol. 4 : 305-313

-----, 1951. The study of instinct. Clarendon Press, Oxford.

-----, 1955. Psychology and ethology as supplementary parts of a science of behaviour. In: First Conf. on Group Processes. B. Schaffner, (ed.), Macy Foundation, New York, 75-167

-----, 1958. Curious Naturalists. Country Life Ltd.

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SENSES INVOLVED IN THE VISITING OF FLOWERS BY *CUCULLIA UMBRATICA* (NOCTUIDAE, LEPIDOPTERA)

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Flowers of *Melandrium album* are frequently pollinated by *Cucullia umbratica* L. This heavy moth always lands on the flowers. The process of insertion of the proboscis and the drinking thereafter is described. From experiments it is concluded that senses for perception of the relative humidity of the air are located on the antennae. The moth is able to locate flowers by smell only, but visual orientation seems to be more predominant. Legs and antennae possess contact chemoreceptors, sensitive to sucrose and to chemicals present on the surface of *M. album* flowers. The moth responds after contact of the legs with petal lobes of *M. album* by extension of the proboscis. Inserting behaviour follows only after contact of the proboscis tip with the ligula. The moth appears to be a specialised flower visitor.

In the summers of 1972, 1973, 1974 and 1975 the pollination of *Melandrium album* (syn. *Silene alba*) in the botanic garden of the University of Nijmegen was studied. The most important pollinator was *Plusia gamma* L. Special attention was paid to *Hadena bicruris* Hufn. (syn. *Harmodia bicruris* Hufn., *Dianthoecia capsincola* Esp.), which oviposits into pistillate flowers of *M. album*. A regular visitor and pollinator appeared also to be *Cucullia umbratica* L. Knuth (1898) presents a record of *C. chamomillae* Schiff. as a visitor of *Silene nutans*. *C. umbratica* has been observed visiting flowers of *S. vulgaris* in England (Marsden-Jones & Turrill, 1957), and of *Viola cornuta* (Hart, 1873). In Nijmegen the moth also visited *S. vulgaris* and *Saponaria officinalis*.

Detailed observations on the behaviour of flower visiting Lepidoptera are scarce. The few studies to be found mainly deal with Sphingidae (Knoll, 1922, 1925, Kugler, 1971, Eisikowitch & Galil, 1971, Gregory, 1963, Brantjes, 1973), or Rhopalocera (Ilse, 1928, Knoll, 1922). The only exact descriptions on Noctuidae visiting flowers are presented by Schremmer (1941), on *P. gamma* and by Brantjes (1976) on *Hadena bicruris*.

Preliminary observations showed that the behaviour and sensory reactions of *C. umbratica* deviate from those of the two other members of the family mentioned above. Therefore, it seems worthwhile to present these observations briefly.

FIELD OBSERVATIONS ON THE BEHAVIOUR

The following descriptions are based on careful observations of seven moths done in the field. Flight, which is inaudible to the human ear, seems well directed

and may vary from very fast to nearly hovering. Flowers extending above the plants' level are visited preferentially. This is in contrast to *Plusia* and *Hadena*, which, when visiting flowers, fly at low level between the branches of the plant. Landing is necessary for *C. umbratica* for drinking purposes. After a landing the flower branch sways heavily. Probably the moth lands with some speed, but the movements of the flower may also be caused merely by the moth's weight, which is twice the weight of other visitors such as *Hadena* and *Plusia*. When landing the moth remains hovering until the legs grasp the petals. Thereafter, the moth hangs

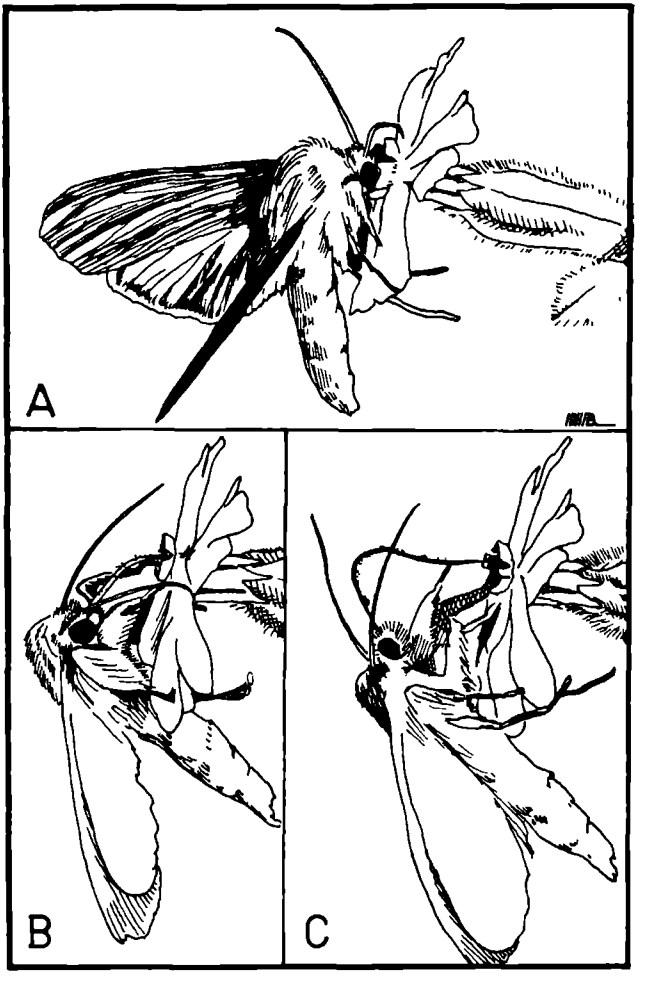

Fig 1 *C umbratica* visits a staminate flower of *M album* A During drinking the wings remain motionless The antennae are kept spread The front and middle legs grasp the petals B After drinking the proboscis is lifted some distance out of the flower Thereafter the moth pushes it back into the tube repeatedly C Just before leaving the flower, the proboscis is lifted out The pollen grains adhering to it are visible (Drawings after photographs)

vertically with its front and middle legs on the petals as is shown in Fig. 1. The wings now stop moving, and are kept somewhat close to the moth's side or are folded rooflike over the abdomen. During flight, the antennae remain spread sideways in front of the insect. After landing the moth moves the antennae towards the flower. The proboscis is unrolled as soon as the legs contact the corolla, and the antennae are kept parallel with the large loop of the proboscis. The entire surface of the corolla is probed with fast movements by both the antennae and the tip of the proboscis. As soon as the tip of the proboscis touches the ligula, the tongue is pushed down, and is guided into the tube by the funnel shape of the flower entrance. During this downward movement of the proboscis and the drinking thereafter, the antennae are kept in a position between sidewards and upwards (Fig. 1A). During drinking, the head remains several millimeters from the flower opening. This is explained by the fact that the length of the proboscis (19, 20, 22, and 22 mm as measured in four insects) exceeds that of the flower tube (18—20 mm). After some time of sitting motionless drinking, the moth withdraws the head several millimeters from the opening (Fig. 1B) and thereafter pushes it towards the flower. This pumping action is repeated for some time. Then the moth lifts the proboscis (Fig. 1C) and leaves the flower. The pumping is probably a search for nectar remains inside the flower.

LABORATORY EXPERIMENTS

Materials and methods

Experiments on sensory capacities were carried out in a cage (60 × 60 × 60 cm) in the laboratory, at very low illumination. The experiments were done during the evening with four specimens one day after capture. Two were caught on flowers, the other two while sitting on an illuminated glasshouse in the garden. The moths were placed in the middle of the cage, on dry blotting paper. As stimulus, a square of blotting paper (1 cm^2) was kept near the antennae, or touched them, or touched the legs. This paper was either dry, moistened with distilled water or with a 20% sucrose solution. The extension of the spiral proboscis within 4 sec after application of the stimulus was called a positive response. Absence of visable reaction or a moving away from the paper was considered a negative one. The number of responses in several tests of each experiment is presented in parentheses after each statement.

Humidity perception

After confinement in the cage for one day the animals reacted to a moistened paper near the antennae (+7, -1). Care was taken not to touch the antennae. Cutting off the antennae until just 3 mm was left did not harm this reaction (+5, -0). After cutting off the whole antennae no reaction was shown (+0, -6). In animals with intact antennae, dry blotting paper did not elicit a reaction (+0, —6), nor did a dry paper, kept between the animal and the moistened paper (+0, —3). We can

conclude from these observations that the insect possesses a sense for humidity of the air located in the antennae. Contact with the tarsus of the front leg was not successful as long as the antennae were protected with dry blotting paper or were cut off (+0, —6). Therefore, the sense for humidity is located exclusively in the antennae.

Contact chemoreceptors on antennae and legs

Animals were allowed to drink water until satiation. Thereafter no response to humidity or contact with water was shown (+0, —6). A positive reaction to sucrose solution, contacting the antennae (+5, —1) or the legs (+11, —1), remained. Both the front and middle leg possess the ability to detect sucrose.

Flowers of *M. album* offered to the moth from a distance long enough to prevent contact with the antennae, failed to elicit reactions (+0, —7) in the water- satiated moths. It has been demonstrated, in experiments described below, that the moth is able to perceive the flower scent. Therefore, we must conclude that the scent alone does not provoke extension of the proboscis. On the other hand, contact of the antennae with the flower caused a prompt extension (+8, —0). An isolated petal lobe turned out to be sufficient for this reaction (+1, —0). After the removal of the antennae, contact of the tarsi with the flower caused despiralisation (+1, —0). From this we conclude to the presence of taste organs, sensitive to *M. album* flowers in both the antennae and the tarsi of *C. umbratica*. The surface of the flower has some stimulating value, additional to the scent, which might be responsible for the proboscis extension by the moth while after landing on a flower.

Contact chemoreceptors on the proboscis

The entrance of flowers of *M. album* is bordered by outgrowths of the petals, which are called ligulae or, together, a corona. To the moths the ligula possesses a signal function of its own, which can be seen in the field and experimental cage. As soon as the proboscis tip touches the ligula the moth pushes the proboscis and the head foreward. This signal function of the ligula was clearly demonstrated by a moth hanging under a flower with its head underneath the brim of the corolla (Fig.

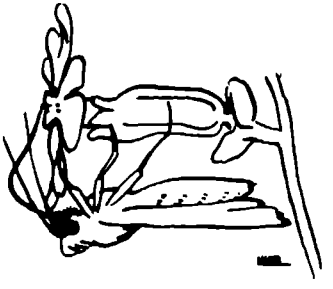

Fig. 2. *C. umbratica* hanging under a flower of *M. album* and trying to insert the proboscis. Each time the tip of the proboscis contacts the ligula the proboscis is stretched and passes along the surface of the petals.

2) The moth was unable to see the entrance of the flower, so the antennae were moved parallel to the surface of the corolla. The proboscis was kept in a large loop between the antennae and the tip probed the entire surface. Each time the ligula was touched by the tip, the proboscis was stretched, but it passed the ligula parallel to the surface of the corolla. This action was repeated several times A flower was offered to another moth hanging on a piece of paper, and it was probed in the same way. After cutting off three of the petal lobes, leaving two lobes on one side, the opening, lined with the ligulas, was left at the edge (Fig 3B). Now the

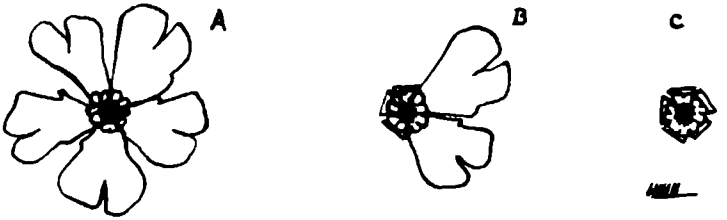

Fig 3 Frontal view of a staminate flower of *M album* a) Intact flower, b) after removal of three petal lobes, leaving the entrance of the flower excentrically, c) After cutting off all petal lobes

proboscis was only pushed foreward after contact with the entrance on the side of the flower (+2, —0). Even after removal of all five petal lobes, leaving on the flower only the claws with the ligulas on top, the proboscis entered the flower tube perfectly (+2, —0). Isolated petals, without ligulas, were probed for about one minute, but they never provoked a "push". Thus *C umbratica* possesses a sense in the tip of the proboscis which can distinguish the ligula from the lobe after contact The curious corona, therefore, has some function as a nectar guide, (cf *Hadena*, Brantjes, 1976).

Smell

The fragrance of flowers is of significance to the moth. This is clearly demonstrated in the following experiments. At sunset two boxes were put on top of a table, standing in the centre of an empty glasshouse. Each box had an opening of 5 × 5 cm in two opposing sides. One box contained 200 flowers of *M album* To exclude the possibility of humidity-directed orientation, the other contained moistened blotting paper along the walls. Two moths, which were put in the glasshouse the night before, were at rest. After half an hour one started to fly and the second moth began one hour later. Both flew towards the table in a zig-zag movement about 30 cm above the ground. In front of the table they rose with irregular zig-zags. One moth landed on the table but continued to fly after one minute. Both approached the entrance of the box containing the flowers, landed on the brim of the opening and walked into the box. The scent of *Lonicera periclimenum* elicited a similar positive attraction. From the flight track a chemotropic orientation seems probable, but anemotaxis cannot be excluded

because there is always some turbulence in the air in a glasshouse. It is therefore possible for the moth to locate source of the odour in the absence of visual clues.

Vision

In the experiments in the glasshouse with hidden flowers, the approach of *C. umbratica* towards a source of scent took much more time than the other moths, flying simultaneously, viz. *H. bicruris* and *P. gamma*. In the field *C. umbratica* approached flowers as fast and direct as other moths did. This may indicate that in normal situations orientation towards flowers is visual or anemotactic. In the field, vision can also play an important role as was shown by Kennedy & Marsh (1974) for male moths orienting towards calling females. But here the surroundings rather than the flower itself will be used by the moth. As was demonstrated before, the flower opening is located by taste, and vision does not seem to be involved.

SOUND PERCEPTION

Although this sense is not directly involved in the behaviour of visiting flowers, it is worthwhile to stress its high sensitivity. *C. umbratica* was much more sensitive to noise than was *H. bicruris* or *P. gamma*. Observations were hindered by this capacity. In the field even the sound of a seed capsule, squashed by the foot of the observer caused the moths to disappear. In the laboratory, after a soft noise the moths remained stationary, but after some time continued activity. Loud noises caused an escape behaviour of the moths visiting flowers. The extreme sensitivity of some Noctuids to supersonic cries of bats is well known, but the sensitivity to low frequency sounds is an interesting phenomenon.

DISCUSSION

It was surprising to find a well-developed sense for humidity in *C. umbratica*. The existence of this sense in adult Lepidoptera has not been reported before. A sense of smell exists, and orientation by smell alone seems possible.

In normal situations, however, scent probably acts as a releaser of anemotactic or visual search for flowers, in the way it has been shown for other moths (Brantjes, 1973, 1976; Schremmer, 1941). After landing, smell alone ceases to function. This can be concluded from the fact that hungry moths, which accepted sugar solution at direct contact, did not react with proboscis extension to the odour of *M. album*. During the visits to flowers, contact chemoreception (taste) is used most and vision is not important. Taste of sugar is more often found to be located on the antennae and tarsi of Lepidoptera. Here we found that the surface of the flower was a stimulant for the extension of the proboscis, while contact of the proboscis with the ligula effects the inserting movement. The nature of the stimulating substances or structures on petal lobe and on the ligula is unknown.

From these specialised reactions, together with the length of proboscis, we can conclude that *C. umbratica* is a well-equipped visitor of flowers. It is an effective

pollinator of *M. album*, and probably of many other flowers. Visits to *Lonicera* can be expected, because the moths orientated to its fragrance, and the proboscis length is sufficient to reach its nectar. In comparison to other moth species, *C umbratica* may be an important pollinator in the Nijmegen area because it is rather abundant.

RESUME

STIMULI SENSORIELS INTERVENANT DANS LA VISITE DES FLEURS PAR CUCULLIA UMBRATICA *(NOCTUIDAE LEPIDOPTERA)*

Les fleurs de *Melandrium album* sont polinisées par *Cucullia umbratica* qui butine ces fleurs apres s'y être posé Les expériences rapportées ici témoignent que des organes sensoriels aptes à percevoir l'humidite de l'air sont localisés sur les antennes Le papillon peut localiser les fleurs par simple odeur mais l'orientation visuelle semble prédominante Les pattes et les antennes possedent des chemorecepteurs de contact, sensibles au sucrose et aux composes chimiques associes aux fleurs de *M album* Le papillon, apres contact de ses pattes avec les petales de cette fleur manifeste une extension de son proboscis et ce n'est qu'après contact de ce dernier avec la ligula, que se realise l'insertion dans la corolle Ce papillon semble être spécialise comme visiteur de fleurs

REFERENCES

BRANTJES, N B M 1973, Sphingophilous flowers, function of their scent In N B M BRANTJES, & H F LINSKENS, (Eds), Pollination and dispersal *Publ Dept Bot Univ Nijmegen* 27—46

——— 1976, Riddles around the pollination of *Melandrium album* (Mill) Garcke (Caryophyllaceae) during the oviposition by *Hadena bicruris* Hufn (Noctuidae, Lepidoptera) *Proc Kon Ned Akad Wetenschap C* **79**, 1—12

EISIKOWITCH, D & GALIL, J 1971 Effect of wind on the pollination of *Pancratium maritimum* L (Amaryllidaceae) by hawkmoths (Lepidoptera, Sphingidae) *J Anim Ecol* **40** 673—678

GREGORY, D P 1963, Hawkmoth pollination in the genus Oenothera *Alisa* **5** 357—419

HART, W E 1873 Fertilisation of *Viola tricolor* and *V cornuta Nature Lond* **8** 244—245

ILSE, D 1928 Über den Farbensinn der Tagfalter *Z vergl Physiol* **8** 658—692

KENNEDY, J S & MARSH, D 1974 Pheromone-regulated anemotaxis flying moths *Science* **184** 999—1001

KNOLL, F 1922 Insekten und Blumen, III Lichtsinn und Blumenbesuch des Falters von *Macroglossum stellatarum Abh Zool Bot Ges Wien* **12** 121—378

——— 1925 Lichtsinn und Blütenbesuch des Falters von *Deilephila livornica Z vergl Physiol* **2** 529—380

KNUTH, P 1898 *Handbuch der Blütenbiologie* Engelmann, Leipzig

KUGLER, H 1971 Zur Bestäubung grossblumiger *Datura*-Arten *Flora* **160** 511—517

MARSDEN-JONES, E M & TURRILL, W B 1957 *The bladder campions* Bernard Quaritch, Ltd , London

SCHREMMER, F 1941 Sinnesphysiologie und Blumenbesuch des Falters von *Plusia gamma* L *Zool Jb Syst* **74** 375—435

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ENTOMOLOGY

RIDDLES AROUND THE POLLINATION OF *MELANDRIUM ALBUM* (MILL.) GARCKE (CARYOPHYLLACEAE) DURING THE OVIPOSITION BY *HADENA BICRURIS* HUFN. (NOCTUIDAE, LEPIDOPTERA), I

BY

N. B. M. BRANTJES

(Communicated by Prof. J. DE WILDE at the meeting of September 27, 1975)

SUMMARY

Larvae of *Hadena bicruris* are seed-predators on *Melandrium album*. The adults use the flowers not only to feed on the nectar but also to oviposit. Both activities are described in detail. For oviposition, which takes place exclusively into pistillate flowers, an extendible ovipositor is inserted into the flowertube. The egg is mostly attached to the ovary. Scent provokes the moth to perform a characteristic "seeking-flight". Apart from the visual means the moths are also able to locate flowers by the smell alone. In the first instance the moth is attracted and then decides by the smell whether or not to land. Tests show the number of landings on objects is proportional to the intensity of the scent. Extracts of staminate as well as of pistillate flowers receive the same number of visits and, therefore, apparently have an equal intensity of odour. Neither calyx, stigma, ovary or the amount of nectar in the flowers are significant for distinguishing between pistillate and staminate flowers by the ovipositing moth. However, the petals, the ligula as well as the lobe, are important. Selection of the oviposition site may be determined by the smell alone. There exists a quantitative relation between the number of petals and the probability to receive eggs. The feeding behaviour and the oviposition behaviour are interrelated and appear as one complex behaviour. The oviposition act is always preceded by drinking from the flower, which becomes pollinated in the process. When the drinking is prevented by satiation of the month, the selectivity with regard to oviposition site is lost. The perception of the flower type takes place in the time interval between the finding of a flower and the insertion of the proboscis. Associations, formed during drinking, influence the selection of oviposition site. However, the inborn preference to oviposit into *M. album* flowers is stronger than known associations with other flower species. Nectar consumption is necessary for a steady egg production. One moth consumes about 23 μl. each night. To obtain this quantity, approximately 100 flowers have to be visited. A pair of moths will pollinate up to a maximum of 500 pistillate flowers, during their life time. The progeny of one pair of moths can destruct more than 500 seed capsules. Therefore, *H. bicruris* is not a symbiont but a real predator of *M. album*.

INTRODUCTION

Larvae of the former genus *Dianthoecia* B. eat almost exclusively from flowers and developing seeds of the Caryophyllaceae. In this respect, they form an exception in the family of Noctuidae, where all species are

leaf-eaters (IMMS 1964). Recently the *Dianthoecia* species have been attributed to the related genera *Harmodia* Hb., *Hadena* Schrk., *Epia*, *Polia*, and *Mamestra* Hb. (KOCH 1958, LEMPKE 1964). According to literature (BLASCHE 1955, KOCH 1958, SEPPÄNEN 1970, SPULER 1910, MARSDEN-JONES and TURRILL 1957, WHITE 1873, WILDE 1861), each species has been observed eating from the seeds of several flower species, mostly within the subfamily Silenoidae. Yet each moth species seems to be associated with a specific combination of food plants.

The described research is restricted to one insect species (*Hadena bicruris* Hufn.). Around The Hague, Professor Doctor L. van der Pijl observed that this moth oviposits on the dioecious *Melandrium album* Garcke, exclusively inside the pistillate (=female) flowers. How does the moth distinguish between pistillate and staminate flowers? This question forms the main problem of this study.

Both male and female adults have a long proboscis and visit flowers for nectar. Does the moth restrict visits to Caryophyllaceae, or, are other species visited in addition? No information has been published on this subject. From my observations of *H. bicruris* it seems very probable that many non-Caryophyllaceous flowers are attractive to the moth but field data is still needed. In general studies on the visits of Lepidoptera to flowers are rare. The only study about Noctuidae was made by SCHREMMER (1941), who studied Autographa gamma L.

During the visits of *H. bicruris* the flowers are pollinated. Because pollination occurs in combination with eventual seed destruction by the caterpillars MÜLLER (1873) and later OLBERG (1951) see an analogy between the symbiotic relationship of *Tegiticula yuccasella*, the yucca-moth, with *Yucca* and *Hadena* with *Silenoidae*.

Is Hadena a plant-predator or a plant-parasite or a symbiont in the sense of SOUTHWOOD (1973)?

Since the moth is destructive to the plant, the benefit might be only to the moth. Olberg does not report how many flowers are pollinated. Stating that all cooperation probably started from a predatory relationship he regards the *Hadena*-Caryophyllaceae relationship to be evolutionarily halfway to a symbiosis profitable to both. Questions about the nature of the relationship will be discussed.

MATERIALS AND METHODS

M. album was grown in the botanical garden of the University of Nijmegen, both for purposes of moth breeding and behaviour observations.

For observations on behaviour and for the experiments on scent attraction in the laboratory, an experimental cage was used. The cage (60 × 60 × 60 cm) consisted of a roof and three walls of white cloth, stained with black dots to raise the visibility of the walls. Before each experiment these walls were moistened with distilled water to keep the relative humidity constantly above 95%. The temperature remained between 19°

and 23°. A movable glass screen in front allowed manipulation and observation. A transformer was used for regulating the light intensity of an incandescent light, projecting a light spot of 10 cm diameter on the center of the right wall.

For the oviposition experiments several small (50 × 25 × 25 cm) experimental cages were used. Two sides and the top were made of netting. The other two sides were of movable glass.

A "pipet-flower", Fig. 1, was made from a pasteur capillary pipet and a square, 15 × 15 mm, cloth of white cotton glued with arabic gum on the opening. Before glueing, a hole to insert flower parts is punched in the center of the cloth with an ordinary paper-punch. Paper provides insufficient grip for the moth after landing. The shape of the piece of cloth, whether round or square, is unimportant, since both forms obtain the same number of eggs. At a construction in the pipet 15 mm from the opening, a glass bead 5 mm in diameter was placed. The hole on the bead filled with 1 μl 20% sucrose solution may act as a nectary.

In each oviposition-preference experiment, one female moth was offered two alternatives at the same time. The distribution of the eggs among the alternatives is regarded as a measure for their relative attractiveness for oviposition.

An alternative consisted of a number of pipet-flowers, each with the same combination of flower parts in the opening. The two alternatives offered, were of the same number of pipet-flowers, which were alternatedly placed in a test-tube rack, separated by a distance of 4 cm.

As will be mentioned in the description of the behaviour, between two depositions the moth always flies for some distance. Consequently the depositions can be regarded as a series of independent decisions made by the moth. Therefore, in case of no preference, the distribution of the eggs over the two alternatives will be a binomial distribution, with expectance 0.5 for each alternative. One alternative is regarded more attractive when it receives significantly more eggs as calculated with the binomial test (SIEGEL 1956), than the other.

FIRST OBSERVATIONS

Life cycle

H. bicruris is active during the whole flowering season of *M. album*. Eggs have been collected from May 21 (1973) until September 18 (1973), while caterpillars have been collected as late as the end of October.

Most of the larvae were collected from infected seed capsules around Nijmegen, and were fed with seed capsules until maturity. Therefore, the animals originate from the local population. In the field the first, the second and the third instar larvae remain inside the ovary and eat the developing ovules. Infection by young caterpillars is recognizable by red feces extruding through a hole in the side of the seed capsule. After the

capsule is emptied, the caterpillar walks over the stem to another capsule and penetrates it.

The last instar larva inserts only its head through a selfmade hole and eats the ovules, leaving its body outside. In the environment of Nijmegen the moth was observed eating the seeds from *Melandrium album* (Mill.) Garcke, *M. rubrum* (Weig.) Garcke, and *M. noctiflorum* (L.) Fr. Sometimes these species are regarded to belong to the genera *Lynchnis* or *Silene*.

On *M. album* each caterpillar destroys about five capsules. Pupation takes place at a depth of 2–5 cm in the soil, and emergence from the pupae occurs about 3 hours before sunset. Both female and male adults begin feeding during the first night, and their life expectancy is about 10 days. Copulation can take place from the second night onwards, the females can only be fertilised once. Eggs are produced only by females which have fed the previous night, and she has be observed to produce 40 eggs in one night, although the average is 20. Usually oviposition starts on the third night. Therefore in the oviposition experiments it is necessary to use "trained moths".

Trained moths are defined as moths that have had a rewarded visit to a scent source.

Selective oviposition

First we have to verify the selection by the moth of pistillate flowers of *M. album* for oviposition. Flowers were collected from the plant's natural habitats in Deurne and Oploo and from the University garden in Nijmegen. The eggs in these flowers were counted (Table 1). It is clear from this that the moth has an absolute preference for pistillate flowers for oviposition.

TABLE 1

Distribution of the egg in flowers of *M. album*.

Flowers, that have been open for at least one night were collected from plants growing in the field. In Deurne and Oploo, both located in Noord-Brabant in the Netherlands, the plants grow along roadsides. In Nijmegen the plants were collected from the botanical garden of the university.

Location	Date	No. of flowers		No. of eggs in the flowers	
		pistillate	staminate	pistillate	staminate
Deurne	1.7.72	42	29	53	0
Oploo	1.7.72	37	19	3	0
Nijmegen	1.6–1.9.72	483	267	29	0

Why does the moth oviposit exclusively inside pistillate flowers? This question can be split up in two parts: How or in what manner does the moth distinguish between the two types of flowers? And what does the moth gain by doing so? The first question will be answered in the

experimental part of this study. The second query can be clarified by the following considerations.

Firstly the ovules form the major food of the larvae. Deposition of the eggs into pistillate flowers guarantees the supply of food to the larvae. Secondly, larvae originating from eggs deposited in staminate flowers have a low chance of survival, although they can eat from these flowers. This low survival rate is illustrated by the following observations. In their third blooming night, staminate flowers drop from the plants. When kept at 17°C the larvae of *H. bicruris* emerge five days after oviposition and can live without food for three days. Therefore, emergence will take place several days after the dropping of the flowers.

Most dropped flowers including the eggs are eaten by snails and other animals within a few days. Although the whole plant is covered with hairs, first instar larvae can climb the plants and penetrate into the capsules. To check this, 25 staminate flowers were provided with five day old eggs and placed on the ground under 20 plants in an insect-free greenhouse. The pistillate flowers on the plants were pollinated artificially to provide food. The egg-laden staminate flowers remained under the plants for at least four days, so all the eggs had an opportunity to develope. Two weeks later, the plants were controlled. Two larvae were present on staminate plants eating from flowers and buds. Two other larvae were on pistillate plants. Obviously these four caterpillars succeeded in climbing up the plants into the flower branches. They had chosen the plants indiscriminately of their sex. Thereafter, as a control, eggs were put in artificially pollinated pistillate flowers left on the plants. After two weeks about 90% of the individuals survived. Thus in this greenhouse mortality of eggs put in staminate flowers is seven times that of eggs put into pistillate flowers. In the field the circumstances may be regarded more severe because most of the fallen flowers are eaten before the eggs finish their development. Therefore, most of the eggs put into staminate flowers can be regarded as lost. In a given population staminate flowers always outnumber pistillate flowers (MULCAHY 1967, 1968). Thus random oviposition would result in the loss of more than half of the eggs. In this respect, the ability to distinguish between the two types of flowers is of great importance to the moth for its survival.

Flight behaviour

In the night, unsatiated moths sit on the wall of the experimental cage or fly around. In the absence of flowers, flight is a circling movement in the upper half of the cage or along the illuminated wall of the cage. Introduction of flowers, visible, or hidden in a box with an opening, causes a change of the flight pattern and also initiates the flight of stationary animals. The flight now becomes what I call "seeking flight". It can be characterised by large sideward loops and sudden drops with slow rises thereafter. It fits the description of the "Nahrungsflug" as has been

described by SCHREMMER (1941, p. 393) for *Autographa gamma*. It is clear that scent from the flowers provokes this change in behaviour.

Drinking behaviour

Both male and female moths drink from staminate as well as pistillate flowers and their behaviour is similar. In their approach of the flower the antennae are kept spread. At the moment of landing, the antennae are moved foreward to each other and pointed downwards, touching the corolla surface. After landing, the proboscis is unrolled and kept in a large loop. With the tip, fast "tapping" movements are made on the surface of the flower. The antennae are mostly parallel to each other, on each side of the proboscis loop. Movements to the sides, however, are also frequently observed. The moth obviously surveys the flower, seeking the entrance. Mostly the tip of the proboscis touches the inner side of the throat of the flower within two seconds, and the proboscis is pushed deeper into the flower-tube. At the same time, antennae are spread and often moved backwards. Once the tongue is inserted, nectar is consumed. The head is kept at a distance of several millimeters from the throat. The moth sits quietly and the wings do not move but sometimes tremble, as during preflight warm-up. After some time the head is pressed into the flower throat, thereafter the proboscis is withdrawn about 10 mm and inserted again with a short nod of the head. Withdrawal and successive insertion are repeated several times. Presumably this pumping is a searching for remains of nectar in the flower. After several pumping movements the proboscis is liften out of the flower tube and coiled again. This marks the end of the drinking behaviour. Immediately thereafter the male moth leaves the flower and flies to another flower.

Oviposition behaviour

For the female moth two lines of behaviour are possible after drinking. The female may fly to another flower or may start oviposition behaviour. Such activity starts with raising the body by stretching the legs and a lifting and fast movement of the wings. The moth now advances several steps over the flower, until it sits with its head above the flower rim. Now the antennae are bent down and pressed onto the petals and move under the moth and become bent backwards. The abdomen, now above the center of the flower, is curved down and the tip is repeatedly pressed onto the petals. Sometimes the ovipositor is extended somewhat. The ovipositor tip, after some trials, enters the flower tube and several changes occur simultaneously. The antennae are raised and spread. Sometimes the wings stop moving. The moth tears at the petals with its legs and rotates the abdomen about 30° to either side. In this way the abdomen is forced down until half of it slips into the flowertube, Fig. 2. Thereafter, the wings stop moving and the telescoping ovipositor is extended to its full length as can be seen in the pipet flower, Fig. 1. The last segment of the

ovipositor has soft parts and is narrower than the diameter of the eggs. This last segment becomes widened locally by the eggs as it passes the ovipositor, a few seconds after full extension of the latter. The eggs adhere to the part of the flower, which contacts the tip of the ovipositor. Within seconds after the deposition of the egg, the ovipositor is withdrawn and the moth leaves the flower.

The start of oviposition behaviour, irrespective of the situation, always leads to the production of an egg. In cases where the opening of the flower is not found by the abdomen tip, the egg is glued somewhere else on the flower. The only cases of interrupted oviposition behaviour are observed in cages without any flowers, where oviposition may only occur somewhere on the wall of the cage. Even in this situation ceasing oviposition behaviour before the eggs are deposited is rare. Only in this situation moths do not drink before oviposition, but normally oviposition is preceeded by feeding activity.

Position of the eggs on the flower

In a flower the eggs are found on the outside of the ovary, on the top as well as at the base, but mostly half way, as is drawn in figure 3. The

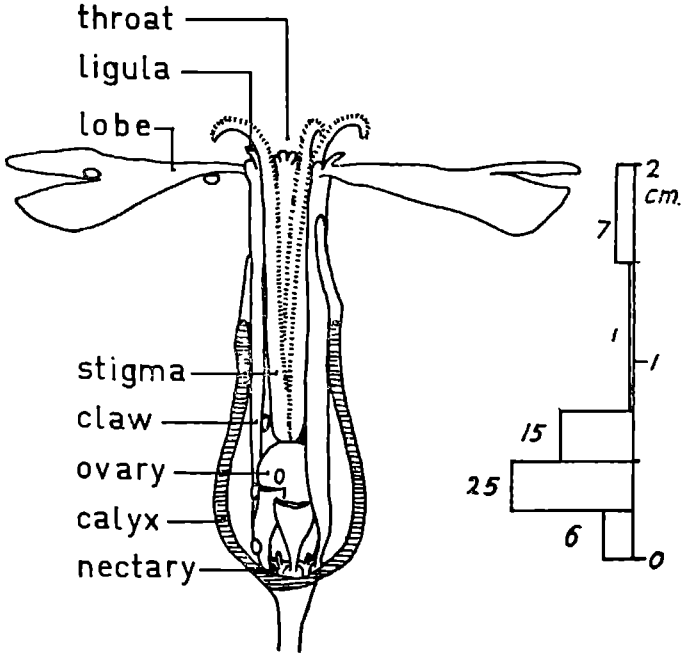

Fig. 3. Positions of the eggs in a pistillate flower. The depth at which 54 eggs have been found in flowers collected in the field is shown in the histogram. The positions where most of the eggs are deposited are shown in the drawing of the flower. The flower is opened by removing the front half of the calyx bladder, two petals and two stigma lobes.

overlapping claws of the petals form a tube. At the base, the tube is closed by the ovary. Since most of the eggs are outside this tube, the ovipositor must be forced between the claws. As can be seen in figure 3, most eggs are at a distance of 18 mm from the throat. The distance corresponds to the length of the ovipositor plus the part of the abdomen inserted into the flower tube. It is concluded that the moth always extends the ovipositor to its full length. This has also been confirmed by direct observations.

Experimental observations

Orientation towards the flower

In order to see which senses are involved in orientation of the insect to the flower, the following experiments were designed.

Isolated flowers of *M. album* were placed into a glass tube (length 5 cm and diameter 2 cm). The tube was open at one end and the opening was covered by 1,0 mm netting. A tube containing a flower and a tube with wetted blotting paper as blank were simultaneously suspended in the centre of the experimental cage. Trained as well as untrained moths approached only tubes containing flowers and always landed on the netting. After landing the proboscis was unrolled and the moth probes through the netting. Flowers hidden in a box with only one opening were also approached by both trained and untrained moths. From this we conclude that orientation only based on scent is possible in this situation.

The capability of learning is clear from the next observation. Fresh moths did not approach white, scentless objects. After a number of visits to flowers moths also approached these objects, cotton or paper, but at a distance of about 2 cm from the object the moth turned and flew away. Flowers behind a glass window were also approached but the moths did not land on it. We can conclude that after training, visual orientation can replace to a large extent olfactory orientation, but scent remains important for landing. In the field the moth will probably rely on both means of perception. The faster, visual orientation serves for the approach to the flower from a distance; the flower scent directed orientation for the last few centimeters.

The attraction value of different flower scents was tested with the glass tubes described earlier. Table 2 shows that a wide variety of flower scents were able to attract *H. bicruris*. The moth was even attracted to the scent of flowers in which nectar is hidden too deeply in the flower tube to be reached by this moth, e.g. *Nicotiana longiflora*.

Can the attraction component be isolated from the flower? Three extracts of petal lobes of *M. album* were made. One with 96% ethanol, one with 100% methanol and one with heptane. For testing, extracts were soaked in strips of blotting paper. After evaporation of the solvent, the strips were introduced into the cage. The number of visits by an untrained moth to each strip is used as an indication of the attractiveness

TABLE 2

Attraction by the scent of flowers.

In each test a glass tube containing an isolated flower is presented simultaneously with a glass tube containing moistened blotting paper. Each "untrained moth" is used only once. After each test the fitness of the moth is controlled with a tube containing a flower of *M. album*. In case the moth does not react positively to *M. album* the test is discarded. One test lasts 15 min. during which the moth flies for at least 10 min. Only tests in which the moths lands on the netting and probes with the proboscis through the netting are positive.

Sex of the moth	No. of positive tests			No. of negative tests		
Flower species	female	male	total	female	male	total
Melandrium album	3	4	7	0	0	0
M. rubrum	0	1	1	2	2	4
Saponaria officinalis	1	–	1	4	–	4
Silene nutans	1	2	3	1	0	1
Dianthus fragrans	0	0	0	1	0	1
Datura stramonium	1	0	1	2	1	3
Nicotiana longiflora	5	2	7	1	0	1
Petunia inflata	2	0	2	0	0	0
Phlox paniculata	4	1	5	0	0	0
Mirabilis longiflora	0	0	0	2	1	3
Oenothera biennis	0	0	0	2	1	3

TABLE 3

Attractiveness of extracts.

Number of landings with probing of the paper by "untrained" moths of *H. bicruris*. Each experiment is the summation of a series of tests. In each test six fresh strips of blotting paper (1 × 10 cm) were impregnated with extracts of petal lobes of *M. album* collected between 9 and 10 pm. The petals were extracted for 12 hours at 21 °C. The strips were suspended with a distance of 5 cm from the top of the cage. Before introduction of strips into the cage the solvent is allowed to evaporate. The arrangement of the 6 strips differs for each test. One test lasts 5 minutes. Each moth is used only once.

Experiment number		Strip number					
		1	2	3	4	5	6
1	solvent	E e[1])	E	M e	M	H e	H
	volume, µl.	70	70	70	70	70	70
	landings	4	0	4	0	3	0
2	solvent	H	H	H e	H e	H e	H e
	volume µl.	40	40	20	20	40	40
	landings	0		10		0	
3	solvent	H e	H e	H e	H e	H e	H e
	volume µl.	20	20	40	40	60	60
	landings	4		7		18	

[1]) E=ethanol, M=methanol, H=heptane, e=extract from *M. album* 10 µl. extract is equivalent with 1/8 petal lobe.

of the extract. In order to distinguish real visits from other landings, only landings where the moth extends the proboscis were counted. Presumably because of the absence of reward a moth visited only one or two strips. This is an indication of a good learning capacity. All three solvents can extract attractive components, Table 3, Exp. 1. The solvents themselves are unattractive substances.

Is attraction dependent on scent intensity? By applying a different volume of extract to each strip, the effect of the odour concentration was studied. From Table 3, Exp. 2 and 3 it can be seen that objects with the strongest scent obtained a greater number of visits. The relation between relative attractiveness and odour concentration approximate a linear relationship.

The attractive power of the scent of both types of flowers can be compared in this way. Extracts with heptane have been made from staminate and pistillate flowers. In Table 4 we see that there is no difference in attractiveness between extracts of both types of flowers. We are tempted to conclude that the odour of both types of flowers will have equal attractiveness to the moths, but we have to make some reservations as to the composition of the odour we used in the tests. The extracts may differ quantitatively and qualitatively somewhat from the odour emitted by the intact flower.

TABLE 4

Relative attractiveness of pistillate and staminate flowers.

Number of landings, with probing, by "untrained" moths. 40 μl. Heptane extracts from petal lobes of pistillate flowers are applicated to 3 strips and of staminate flowers to three different strips. The six strips are suspended simultaneously in the cage. 40 μl. is equivalent to 6/10 petal lobe. Further details see Table 3.

Night after emergence of the moth	1	2	3	4	5	Total
staminate flower extract	2	7	9	5	4	27
pistillate flower extract	3	6	9	8	3	25
						52

Nectar consumption

How many flowers does a moth visit during one night, and what amount of nectar is consumed? This is determined in the experimental cage by the following experiment: Freshly picked flowers were carefully placed in pipet flowers. Sets of 20 flowers were introduced simultaneously into the cage. After 10 flowers had been visited by a given moth, a new set of 20 flowers was introduced. This was repeated until the moth landed and folded the wings in a rooflike fashion over the abdomen – a position presumed to reflect satiation. In two hours 94 visits were paid to a fresh flower and 68 visits to a flower previously visited. By sucking the nectar from a carefully opened flower into a callibrated glass-capillary the nectar

content of the flowers was determined during the experiment. An average of 0.233 μl per unvisited flower was found and there was no nectar in visited flowers. Therefore, the moth has consumed a total of 22 μl nectar that night.

To determine the amount of nectar consumed by a moth each day, and the influence of the concentration, the following experiment was carried out.

Several moths were fed once a day until satiation during the first six days of their life. For feeding, the moths were encaged in a teflon tube, Figure 4. A needle was placed in the spiral of the proboscis. As the needle was moved in the direction of the sugar solution, it forced the proboscis to unroll. As soon as the tip of the tongue contacted the sucrose solution, the animal stretched the tongue actively and started drinking. After some time the proboscis was withdrawn and spiralised. Consequently the forced unrolling was repeated. As soon as five forced unrollings were not followed

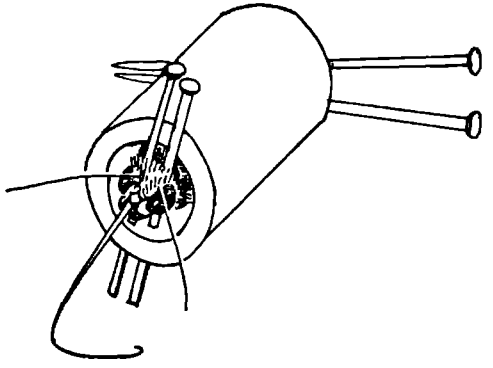

Fig. 4. Teflon tube used to feed *H. bicruris*, The tube (length 15 mm., inner-diameter 8 mm) has two pins through each side. Because of the elasticity of the wall, the pins can be bent outward to introduce the moth. In the crevice between the head and the thorax the pins fix the moth.

TABLE 5

Consumption of sucrose solution

The mean consumption (μl.) per day of a sucrose solution (10%, 20%) by *H. bicruris*. The number of observations is given in parenthesis. The moths are fed once a day until satiation during the first six days of their life. Between two feedings the moths were kept in a moisture saturated cage at 20 °C.

Sex of the moths	Sucrose percentage 10%	20%
male	19.7 (15)	26.7 (12)
female	20.6 (27)	24.7 (33)
total	20.2 (42)	25.2 (45)

by drinking the moth is considered to be satiated. In Table 5 we see that both sexes consume an equal quantity of sucrose solution. The moths drink more sucrose solutions of higher concentration. This is perhaps because stronger taste competes longer with growing satiation. The quantities of the sugar solution consumed in this experiment are a good likeness with the calculated quantity of nectar consumed by moths visiting flowers. This likeness strengthens the earlier presumption that moths cease visiting flowers when satiation is achieved.

What is the importance of the sugar uptake for the moth? To allow several moths to feed freely, the same glass tubes from the experiments on scent orientation were used. In one end of the tube a Petunia flower was placed. The scent of the flower has been proven to be attractive to the moth (Table 2). A small wad of cotton, impregnated with sugar solution, was laid behind the netting. Therefore, after the proboscis pierces the netting, the impregnated cotton is touched, and the moths begins drinking. Twelve moths which were freely allowed to drink from this food source produced 119 eggs per female.

Four moths kept under identical conditions, but with pure water substituted for sugar solution in the cotton, produced no eggs. Therefore, the consumption of sugar appears essential for eggs production. Sugar is also important to life expectancy of the moths. In the experimental situation the mean lifetime of fed moths was 8.7 days and that of starved ones 6.5 days. This difference is significant.

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ENTOMOLOGY

RIDDLES AROUND THE POLLINATION OF *MELANDRIUM ALBUM* (MILL.) GARCKE (CARYOPHYLLACEAE) DURING THE OVIPOSITION BY *HADENA BICRURIS* HUFN. (NOCTUIDAE, LEPIDOPTERA), II

BY

N. B. M. BRANTJES

(Communicated by Prof. J. DE WILDE at the meeting of September 27, 1975)

Characteristics of the flower decisive for the type discrimination

In the field only pistillate flowers receive eggs, as shown in Table 1. Are staminate flowers totally inadequate for oviposition or is it just a matter of preference? When staminate flowers of *M. album* are presented exclusively, moths will oviposit in these flowers. Therefore, the staminate flowers are acceptable for oviposition, but the moths prefer pistillate flowers.

It had to be determined firstly if discrimination capacity for the two types of flowers is preserved only in the experimental situation. Table 6, Exp. 1 and 2, reveals a significant preference for pistillate flowers for oviposition. Reduction of the number of eggs is sufficient for calculation of the significance, Table 6, Exp. 3–7. However, the choice of the moths is not as absolute as in the field. This might be due to the experimental situation which is less favourable to the moth.

There are several possible methods for determining which parts or characters of the flowers are decisive in the selection by the moth. One has only to remove in successive experiments additional parts of the flower until ovipositional selectivity disappears. The flower part removed last might be the decisive part. Another possibility is to interchange parts of one type of flower with parts of another, and to see whether the moth changes its preference. Both possibilities are utilised. Flower parts are placed in a pipet flower as described earlier (materials and methods). The pipet flower itself is not attractive to the moth for oviposition as can be seen in Table 7.

Significance of calyx bladder and of the stigma is determined by removing them from the flowers. Table 8 shows that elimination of calyx (Exp. 1 and 2) or of both calyx and stigma (Exp. 3 and 4) does not change the moths selectivity. The conclusion is that these parts are not essential for the recognition of the type of flower.

Because the egg is deposited usually on the outside of the ovary, and

TABLE 6

Selectivity for oviposition in the experimental situation.

In each experiment flowering branches cut from the plants are offered to one moth in the experimental cage. The number of pistillate flowers equals the number of staminate flowers. After one night the flowers are collected and the eggs are counted.

Experiment number	Total no. of flowers	Total no. of eggs in the flowers		Difference
		staminate	pistillate	
1	100	13	28	15 * [1]
2	100	2	26	24 * *
3	20	0	3	3
4	20	5	6	1
5	16	0	5	5 *
6	10	0	3	3
7	10	1	6	5
		21	77	56 * *

[1]) Significance of the difference, calculated with the binomial test, (*=0.05, **=0.01).

TABLE 7

Attractiveness of pipet-flowers for oviposition.

Pipet-flowers contained parts of freshly cut, pistillate *M. album* flowers. Alternative 1 contained no flower parts. In alternative 2, each pipet-flower contained 5 petals. The two alternatives were presented at the same time during one night.

Experiment number	No. of flowers in each alternative	No. of eggs in alternative		Difference
		1	2	
1	2	0	5	5 * [1]
2	2	0	3	3
		0	8	8 * *

[1]) Significance of the difference, calculated with the binomial test, (*=0.05, **=0.01).

because the moth contacts the ovary also with the proboscis while drinking from pistillate flowers, this flower part has a good chance of being the point of recognition. Moreover, the tip of the proboscis of *H. bicruris* is covered with a characteristic kind of sense organs (BRANTJES 1973b). Ovaries were added to pipet flowers containing staminate or pistillate petals. A bead with sugar was added to the other alternative to compensate for the influence of the nectary associated with the ovary. Table 9, Exp. 1–3, shows the absence of preference to oviposit in flowers with or without ovary. Obviously, the ovary is not the point of recognition.

The amount of nectar present in pistillate flowers usually exceeds the amount in staminate flowers of *M. album*. Because drinking behaviour

TABLE 8

Influence of calyx and stigma on the preference for oviposition.

Pipet flowers contained parts from freshly cut *M. album* flowers. The pipet-flowers did not contain a bead with sugar water. The two alternatives were presented at the same time during one night.

Experiment number	'No. of flowers in each alternative	Flower parts in pipet-flower	No. of eggs in alternative		Difference
			1 pistillate flowers	2, staminate flowers	
1	8	flower without calyx	8	0	8 * *[1]
2	6	ibid.	2	0	2
			10	0	10 * *
3	16	flower without calyx or stigma	18	2	16 * *
4	16	ibid.	3	2	1
			21	4	17 * *

[1]) Significance of the difference, calculated with the binomial test, (* *=0.01).

TABLE 9

Influence of ovary and of sugar water on oviposition preferences.

Parts of freshly cut flowers were placed in pipet flowers. Two alternatives were presented at the same time during one night to one moth in each experiment.

Experiment number	No. of flowers in each alternative	Composition of alternative		No. of eggs in alternative		Difference
		1	2	1	2	
1	12	st [1])	st, ov	10	13	−3
2	12	st, b, s	st, ov	13	6	7
3	6	pi, b, s	pi, ov	8	6	2
				31	25	6
4	10	st, b, s	st, b	4	1	3
5	7	st, b, s	st, b	3	2	1
6	6	st, b, s	st, b	18	22	−4
7	3	st, b, s	st, b	6	2	4
8	3	st, b, s	st, b	0	1	−1
9	3	pi, b, s	pi, b	7	5	2
				38	33	5

[1]) st = five petals of a staminate flower
pi = five petals of a pistillate flower
ov = an isolated ovary with stigmas added into the tube
b = a bead placed in the pipet flower
s = sugar water added to the bead.

always preceeds oviposition and because the moth can be trained to oviposit on its feeding place, it is possible that the moth bases its choice on the amount of nectar present in the flower. By adding sugar water in the bead of one half of the pipet flowers this hypothesis was varified, Table 9, Exp. 4–9. Only a very slight raise in the number of eggs obtained by these flowers was observed. Therefore, the amount of nectar in a flower does not determine the selection. The only remaining possibility is the corolla. Isolated petals were put in pipet flowers in varying combina-

TABLE 10

Signal effect of petals.

Petals of freshly cut flowers were placed in pipet-flowers. All flowers of the two alternatives were presented at the same time during one night to one moth in each experiment. The figure before staminate or pistillate denote the number of petals in the pipet-flower.

Experiment number	No. of flowers in each alternative	Composition of alternative		No. of eggs in alternative		Difference
		1	2	1	2	
1	12	5 pi [1]	5 st	2	5	−3
2	12	5 pi	5 st	5	6	−1
3	6	5 pi+b	5 st+b	6	13	−7
4	3	5 pi+b	5 st+b	5	2	3
5	3	5 pi+b	5 st+b	0	1	−1
6	6	5 pi+b+s	5 st+b+s	0	2	−2
7	6	5 pi+b+s	5 st+b+s	3	1	2
8	6	5 pi+b+s	5 st+b+s	4	3	1
9	3	5 pi+b+s	5 st+b+s	7	6	1
10	6	1 pi+b	1 st+b	2	0	2
11	6	1 pi+b+s	1 st+b+s	5	5	0
				39	44	−5
12	6	2 st+b+s	1 st+b+s	23	13	10
13	6	4 st+b+s	1 st+b+s	8	4	4
14	6	3 pi+b+s	1 pi+b+s	27	5	22 * * [2]
15	6	4 pi+b+s	1 pi+b+s	8	4	4
				66	26	40 * *
16	6	3 pi+b+s	1 st+b+s	8	0	8 * *
17	6	3 pi+b+s	1 st+b+s	29	7	22 * *
18	6	3 st+b+s	1 pi+b+s	13	0	13 * *
19	6	3 st+b+s	1 pi+b+s	24	2	22 * *
				74	9	65 * *

[1]) st=petals of staminate flowers, pi=petals of pistillate flowers, b=bead, s=sugar water added to the bead.

[2]) Significance of the difference, calculated with the binomial test, (*=0.05, * *=0.01).

tions with beads containing sugar water. If the number of petals is 5, Table 10, Exp. 1–9, or is 1, Exp. 10–11, there is no clear preference for petals of pistillate flowers. Since all eggs are laid in the pipet flowers it is clear that isolated petals are directing oviposition. One might, however, conclude that recognition of the type of the flower is not based on the petals. In experiments where only calyx and stigma are removed, Table 8, moths recognised the difference between petals from pistillate and from staminate flowers. In the last experiments, Table 10, Exp. 1–11, the ability to recognise this difference, was lost. The set-up differs only in one respect: the petals were separated from the flower bases in the last experiments. The way in which isolation of petals from the flower causes a loss in preference is not yet clear.

We have seen that the attractive value of scent, for approach and subsequent drinking, is proportional to its intensity. Is the induction of oviposition by petals also related to the number of petals? The number of petals in each alternative was varied, Table 10, Exp. 12–15. Pipet flowers with the majority of petals obtained most of the eggs. The quantity of petals is clearly a determining factor in oviposition. By simultaneously varying the number of petals and the type of the flower, the relative importance of each can be compared (Table 10, Exp. 16–19). The results show that the quantity of petals is more important than the type of the flower from which the petals originate.

A petal consists of a white petal lobe, a colorless claw and at their junction a white ligula. Which part is involved in the selection by the moth of its substrate for oviposition? To determine the importance of the ligula, ligulas were carefully cut with cleaned pairs of scissors. To prevent contamination care was taken not to touch the petal lobes. It can be seen from Table 11, Exp. 1–2, that both pistillate and staminate flowers obtained a smaller number of eggs after removal of the ligula. The presence of the ligula increases the oviposition directing capacity of the flower.

TABLE 11

Influence of the ligula on oviposition.

In each experiment 20 flowers were put in a pipet-flower and introduced in the experimental cage. Of 10 flowers, forming one alternative, the ligula was carefully removed. During one night one moth is allowed to oviposit in the flowers in each experiment.

Experiment number	Flowers alternative		No. of eggs in alternative		Difference
	1	2	1	2	
1	pistillate	pistillate – ligula	18	7	11 * [1]
2	staminate	staminate – ligula	25	8	17 * *
3	staminate	pistillate – ligula	0	3	3

[1]) Significance of the difference, calculated with the binomial test, (*=0.05, * *=0.01).

However, the ligula is not the only determining factor, as can be seen from Table 11, Exp. 3. Although the difference is not significant, due to the small number of eggs, we can safely conclude that pistillate flowers without ligulas obtain eggs in the presence of intact staminate flowers. Other parts of the pistillate flowers, most probably the petal lobes, also have an oviposition inducing capacity.

Senses involved in perception of the flowertype

With which sense or senses does the moth perceive the flowertype? All oviposition experiments described previously were performed in an absolute dark room. Therefore, the decisive factor must be chemical and perceived by smell or by taste. Both possibilities will be discussed here. Let us assume that taste, defined as contact chemoreception, is involved. The parts of the body contacting the flower are the proboscis, antennae, ovipositor and legs. The hypothetical taste receptors have to be located on one of these body parts. In some experiments, Table 10, Exp. 12–15, in the pipet flowers of both alternatives petals were placed, originating from the same type of flower. Here the receptors contacting these petals will obtain the same information in visits to both alternatives. We have seen that selection of oviposition side can be based on the quantity of petals, Table 10, Exp. 12–19. Quantitative variation, therefore, can only be found in the number of receptors contacting the petals. The number of receptors contacting the petals vary only for the legs, because the number of legs contacting the petals can be related to the number of petals offered in the pipet flower. When the insect visits intact pistillate and staminate flowers the number of receptors contacting the petals remains constant. In this situation, the receptors themselves have to perceive a quantitative difference. Thus, if contact chemoreception is decisive, the receptors have to be located on the legs and each receptor has to respond quantitatively to its stimulus. The moth has to be able to react equally to both the number of stimulated receptors and to the quantity of the stimulus. There is no experimental evidence to reject the possibility that contact chemoreception could be involved.

To determine whether smell alone can be utilised by the moth to select the site for oviposition the following experiment was performed.

6 Flowers of *M. album* were suspended upside down above the netting of the cage and separated by a distance of 5 cm. The distance from the petals to the netting was 1 cm, therefore tarsal contact was impossible. In four tests, 15 out of 18 eggs were deposited exclusively underneath the hanging flowers. From this it is concluded that odour alone is sufficient to direct the oviposition. Also observations during feeding show that isolated odour has an oviposition-directing effect. Feeding tubes scented by putting a flower at the back, were put in the cages. The tubes were sealed above by netting and a wad of cotton impregnated with sugar solution was placed below the netting. The surface area of the cage netting

was 0.3 m^2, while the surface of the netting of the tubes was less than 0.003 m^2. The possibility of each egg being deposited on the feeding tube therefore is less than 0.01. There were always significantly more eggs deposited near the tubes than could be expected, Table 12. Yet a large number of eggs were deposited on the wall of the cage. This might be explained by low intensity of the scent emitted from the tubes, or the loss of the scent by the flowers during the night and the resulting random deposition thereafter. From Table 12 it is clear that a diversity of flower scent can direct the oviposition.

TABLE 12

Preference of oviposition near a source of scent.

During one night glass feeding-tubes, (for description see nectar consumption), are suspended in the experimental cage with several moths. In the morning the eggs are collected.

Experiment number	Flower species	No. of eggs deposited	
		1, near the scent	2, on the cage
1	*Phlox paniculata*	6	24 * *[1]
2	*Phlox paniculata*	4	73 * *
3	*Nicotiana longiflora*	7	18 * *
4	*Nicotiana longiflora*	5	53 * *
5	*Nicotiana longiflora*	2	7 * *
6	*Melandrium album*	6	25 * *
7	*Melandrium album*	5	31 * *
8	*Melandrium album*	3	40 * *
9	*Melandrium album*	0	23
10	*Melandrium album*	2	9 * *
11	*Melandrium album*	1	4

[1]) * * = significant deviation from the expected ratio of 1:100, as calculated by the chi-square test with a significance level of 0.01.

Odour from staminate and pistillate flowers

Because the site of oviposition can be selected by smell and because moths select pistillate flowers for oviposition, one may ask whether the odour of staminate flowers differs from that of pistillate flowers. To compare the odours from both types of flowers, odour was trapped with the "enfleurage" method using Apiezon L as grease. In a gaschromatograph, on a column filled with 10% Apiezon L on Chromosorb W, the compounds present in the odour were separated but have not yet been identified. The odours of staminate and pistillate flowers consist of identical compounds in equal proportions. The relative importance to the moth of each component has not yet been tested.

Since the scent of both types of flowers do not differ qualitatively, the only difference can be in the quantity of the scent emitted. From

the finding described previously, Table 4, that heptane extracts from corollas of both types of flowers attract the same number of moths, together with the conclusion that attraction is proportional to the quantity of scent, we conclude that both types of flowers emit the same quantity of odour. These conclusions, that the quality and the quantity of scents of both types of flowers are equal, contrast with the expectance that the pistillate flower emits more scent, at least more of the components important for oviposition when compared to staminate flowers. This expectancy is based on the observation, Table 10, Exp. 12–15, that the moth selects the place of oviposition by smell and prefers flowers with the greater number of petals, therefore the largest scent emission.

Relation between drinking behaviour and oviposition behaviour

In the description of the behaviour it is stated that drinking behaviour always preceeds oviposition. Is this correlation accidental or is it physiologically determined? To test this the drinking behaviour was inhibited by feeding the moths until satiation with the teflon tube technique as described earlier under nectar consumption.

As a result of feeding, egg production continues and moths are "urged" to deposit eggs. To guarantee a high contact frequency of the moth with flowers, satiated individuals were encaged individually, inside a circular corridor with a length of 50 cm and a diameter of 3 cm .In this corridor a pistillate flower was placed. Under the experimental conditions, seven moths deposited a total of 109 eggs in one night. Only two of the eggs were deposited on a flower, on the outside. The rest was dispersed throughout the corridor. One other moth, after drinking a limited amount of sugar water, deposited two eggs, both inside the flower. In all other cases previous satiation inhibited drinking behaviour. Consequently, oviposition inside the flower was not performed. Drinking forms an integral part of the behaviour leading to oviposition inside a flower.

Influence of associations formed during drinking

Because nectar consumption is required for egg production each moth ovipositing inside flowers must have previously drunk from flowers. Several other flower species are also utilised for drinking. The moth may become "trained" during those visits. Training by "association learning" on flowers is possible in the same way as it is shown by SCHREMMER (1941) for *Autographa gamma*. Moreover drinking behaviour and oviposition behaviour are closely associated. Therefore, associations formed during drinking may influence selection of the place of oviposition. The contribution of this association and of inborn factors influencing oviposition must be clearified.

Several moths were offered exclusively flowers of *Phlox paniculata* for feeding. It is a substrate which is normally never used for oviposition. Flowers were cut from plants and supplied with sugar water. Moths drank

from these flowers, and after some days females oviposited into the narrow *Phlox*-flower tube, or on the petal lobes, as shown in Table 13, Exp. 1. By tearing the tube along its length and positioning it into a pipet flower the tube is widened, and becomes a better shape for oviposition. These flowers were then presented to female moths. In this case oviposition was exclusively limited to these pipet flowers with *Phlox* petals, Table 13, Exp. 2. Drinking from these flowers clearly induced the moths to oviposit on these flowers. The association formed during drinking affects the oviposition behaviour. The next day the same moths were offered nectarless pipet flowers containing a petal of *M. album*, together with the pipet flowers supplied with *Phlox* petals and sugar solution.

TABLE 13

Influence of "training" on the selection of plants.

Moths that had been fed exclusively near the scent of *Phlox* flowers are offered several alternatives for oviposition. The experiment lasts one night and is performed in the dark. The pipet-flowers all contain a glass bead. Sugar water is added only to *Phlox*-flowers and to pipet-flowers with *Phlox* petals. A=intact *Phlox*-flowers with on flowering branches cut from the plants. B=pipet flower with petals of *Phlox*, C=pipet-flowers with petals of *M. album*, D=walls of the experimental cage.

Experiment number	No. of flowers in			No. of eggs deposited on			
	A	B	C	A	B	C	D
1	47	–	–	5	–	–	30
2	–	12	–	–	19	–	0
3	–	9	3	–	5	10	0

Many eggs were deposited in these *M. album* flowers, Table 13, Exp. 3. The *Phlox* flowers were devoid of their nectar and therefore had also been visited. The inborn preference for oviposition on *M. album* was stronger than the formed association with the source of food, although a residue of the association was left, as is shown by the 5 eggs deposited in the *Phlox* pipet flowers.

Internal factors affecting selectivity by the moth

Why is selectivity in oviposition not always absolute? In lepidoptera, ovulation is independent of any external stimulus (WIGGLESWORTH, 1972). Mature eggs are stored in the ovariole stalk (EIDMANN, 1970), which gradually becomes filled. We can suppose that over-production of mature eggs causes the "urge" to deposit. This "urge" is often noticeable in behaviour of the moths. Presenting staminate and pistillate flowers to a moth in the "urge" condition results often in a series of eggs being laid within minutes in both flower types, after which the eggs are laid less frequently and with more selectivity, Table 14, Exp. 1. In a normal situation, where flowers are available at all times, eggs are deposited with

TABLE 14

Sequence of visits to flowers and resulting behaviour.

In the experimental cage freely flying female moths were offered freshly cut flowers of *M. album*. In Experiment 1 the moth was captured in the field and encaged for one day prior to the experiment. In the cage were 7 pistillate and 11 staminate flowers. In Experiments 2 and 3 two moths, originating from cultivated pupae were fed artificially in the teflon tube. The moths were tested separately, flying free in the experimental cage. Flowers were introduced one after another in the sequence given in the table, and were removed after a single visit by the moth.

Experiment number		
1	flower [1])	p s s s s s s s p s s p p s p s p p s s s
	behaviour [2])	o d o d d d o d o d d o o d o d o o d d d
2	flower	s p s p s p p p p s p s s s p
	behaviour	d o d o d o d o o d o d d d o
3	flower	s p p p p s s p
	behaviour	d o o d o d d o

[1]) p=pistillate flower, s=staminate flower.
[2]) d=drinking, o=drinking and oviposition.

intervals of 10 to 15 minutes. Presumably in this situation the ovariole stalk is empty and ovulation is followed by oviposition. After a time period without flowers a moth in "urge" will oviposit even on the walls of the cage. A growing "urge" to oviposit clearly lowers selectivity by the moth.

Time of perception of the flower type

Normally moths visit both pistillate and staminate flowers in a mixed sequence when both types are available. Oviposition only occurs in pistillate flowers. Does the moth visit flowers in a random sequence and recognise the pistillate flowers during or after landing, or does the moth direct its flight to pistillate flowers with the intent to oviposit in them? By imposing a given sequence of visits on the moth this is analysed. Two moths were fed artificially via the teflon tube prior to the experiment. These specimens had never visited flowers. In the cage flowers were presented one after another to a moth. The selectivity was perfect, Table 14, Exp. 2 and 3. Clearly the decision to oviposit or not is made after perception of the flower. The moths produced 7 eggs within 15 min; 4 eggs within 10 min respectively. From the shortened lag period between ovipositions we conclude that the moths were in a slight "urge" to oviposit, but it did not hamper selectivity.

On several occasions in these experiments moths inserted the proboscis about 10 mm into the pistillate flowers only for a short time. The moths did not seek nectar in these flowers but immediately proceeded to insert

the ovipositor into the flower. In visits to staminate flowers, before and after these visits to pistillate flowers, the moths inserted the proboscis deep into the flowers. After drinking the moths were intensely seeking nectar remains. Drinking behaviour on staminate and on pistillate flowers clearly differed. Obviously the moths did perceive the type of the flower before the onset of drinking. In more normal situations the moths behaviour did not differ on both types of flowers. The two moths in this experiment were in "urge" condition. Maybe because of this the time available for drinking from the pistillate flowers was reduced in favour of egg deposition. The type of the flower can influence the behaviour beginning with the insertion of the proboscis. The perception of the flower type must occur before this moment. The decision to oviposit or not is made after perception of the flower. Therefore, the moment of recognition of the flower type by the moth occurs between perception of the flower and the inserting of the proboscis.

Pollination

Hadena bicruris visits *M. album* flowers for the purpose of drinking. The effectiveness of drinking in causing pollination was ascertained in the following experiment. In the experimental cage moths were allowed to visit isolated, intact pistillate and staminate flowers. The pistillate flowers originated from insectfree cages and checks showed that these flowers were free of pollen grains.

Stigmas were removed from these pistillate flowers, after one visit. Care was taken to prevent contamination of the stigmas. The stigmas were individually squashed and saffranine was added, only pollen grains are stained by this dye and could be counted easily. During alternating visits to 8 pistillate and 8 staminate flowers the moths transferred an average of 316 pollen grains per visit, the maximum was 605, the minimum was 37. We conclude that pollination of pistillate flowers is fully accomplished by one visit of a moth which has previously visited a staminate flower.

The proboscis is the only part of the moth contacting the stamens and stigmas. Because pollen of *M. album* forms a very lose powder, it will not adhere very well to the proboscis. Therefore, we may question how long pollen remains on the proboscis. The moth was allowed to visit several staminate flowers first to obtain maximum load of pollen on the proboscis. Thereafter, only pistillate flowers were left in the cage. On stigmas of the flowers visited in succession I counted 601, 57, 8 and 3 pollen grains per flower. The number of grains deposited per flower decreases sharply after successive visits to pistillate flowers. Therefore, additional visits to staminate flowers are necessary to reload the proboscis with pollen. The behaviour of the moth fits this condition for maximum pollination. Both male and female moths visit both types of flowers when they are available, as shown in Table 14, Exp. 1.

Pollination, which results in fertilization, of a pistillate flower in which an egg is deposited, is important to secure a food supply for the larvae. We may ask whether pollination can be effected by the ovipositor during the deposition of the egg. To investigate the adherance of pollen grains to the ovipositor two female moths were presented only staminate flowers for oviposition. After several ovipositions the moths were killed. There were no pollen grains on the ovipositor. Therefore, the ovipositor cannot function in pollentransport. Moreover, normally moths never oviposit into staminate flowers, thus ovipositor will never come into contact with the anthers.

Drinking forms an integral part of oviposition behaviour. The coupling of drinking as a pollinating act with oviposition is very profitable to the moth. How well this pollinating act is preserved, even in unnatural situations, is illustrated by the experiments presented in Table 14. In Experiment 1 the moth, in "urge" to oviposit still alternates visits to pistillate and to staminate flowers, and in this way depleating the pollen load on the proboscis is prevented. The moths used in Experiments 2 and 3 sometimes did not drink from the pistillate flowers. The proboscis was pushed between the stigmas only for a short moment. Yet this appeared to be sufficient for transfer of pollen, as was revealed by counting the number of pollen grains on the stigmas: 43, 276 and 222 grains per flower.

Discussion

Is the behaviour of *H. bicruris* comparable to the behaviour of other Lepidoptera? Oviposition preferences in Lepidoptera are genetically determined and often restricted to one host species or to a narrow range of related species. Mostly the selection of plants is based on a chemical factor. This is also the case with *H. bicruris*, which prefers Silenoidae for oviposition, independent of "training". The plant for the oviposition is selected by smell. Eggs have been found also in flowers of *M. rubrum* and *M. noctiflorum* in the field. The scent of flowers of these plants and probably of other Caryophyllaceae as well seems to be effective in induction of the oviposition behaviour. Yet in the botanical garden in Nijmegen where many species of Caryophyllaceae grow together, *H. bicruris* clearly prefers *M. album*. Why *Hadena* species restrict themselves to Caryophyllaceae for larval food is not yet known. The only parts of the plant eaten are the flowers and the developing seeds. It is probable, that these organs have a high nutritional value. The caterpillar will not eat any substrate on which the egg is deposited. The oviposition exclusively inside pistillate flowers of *M. album* agrees well with the feeding preferences of the larvae.

Until the fourth instar stage *Hadena* larvae live inside the seed capsule. This mining habit of *Hadena* is unique in his family, Noctuidae. One advantage is the protection from dessiccation, one of the major dangers to insects living on the outside of plants (Southwood 1972), especially to the young ones with a high surface-to-volume ratio. An additional

advantage for the moth might be the protection offered by the seed capsule against predation. However, this advantage remains to be studied. Anyhow populations in the field are often heavily infested, up to 70%, with parasitic Hymenoptera. The last instar larvae sitting on the outside and eating from the capsule on top of the flower branches is very conspicious, at least to a trained human eye.

The oligophagy of caterpillars and related specificity in oviposition in many Lepidoptera species, contrasts with polytropy of adults. In flower visits some Sphingidae have innate reactions to specific flower scents, but this never excludes visits to other flower species (BRANTJES 1973). Less selective flower visitors are also found within the Sphingidae (KNOLL 1925, KISLEV 1972). For the Noctuidae SCHREMMER (1941) discovered that *Autographa gamma L.* has no innate specific preference in flower visits. Preferences can develop during the life of a moth and are determined by training. In my experiments this has been found also to apply to the feeding preferences of *H. bicruris*. Moths are attracted by a diversity of flower scents and the preferences are susceptible to experience.

Both oviposition and visits to flowers by *H. bicruris* can be compared with those of other Lepidoptera, but one aspect remains unique for *H. bicruris*. For all other Lepidoptera both activities are separated, but for *H. bicruris* and presumably also for its near relatives, both activities are connected in one complex behaviour pattern. This fixed combination has very important consequences for the moth. Pollination results in food for the young, being available at the right time. This offers an important survival value for the species. The only analogy in the Lepidoptera discovered until now is the relation of Yucca moth with Yucca flowers. This relationship is a form of real symbiosis, with benefit both for moth and for plant.

Is the relationship of *M. album* and *H. bicruris* also profitable for both partners? A simple calculation may answer this question. The profit for the plant is the pollinating work done by the moth. To become satisfied a moth has to drink 22 μl nectar each night. In the experiment on nectar consumption a moth visited 94 "virgin" flowers, each containing 0.23 μl nectar, to obtain this quantity. Flowers with higher nectar contents (up to 2 μl) have been observed in the field. If this is the case each moth will visit even less flowers. We will assume that 100 "virgin" flowers are visited by one moth during one night. MULCAHY (1968) states that in the field there are between 2 and 6 times more staminate than pistillate flowers. My observations in general agree with his. Therefore, if for drinking the moth selects both types of flowers indiscriminately an average of 25 pistillate flowers from 100 flowers are visited for the first time and are pollinated. A male and a female moth, with a life span of 10 days, together will pollinate 500 flowers. The loss to the plant is the destruction of the seed capsules by the larvae. Since larvae destroys approximately five capsules each, and one pair of moths produces more than 100 eggs,

we calculate a loss of more than 500 capsules caused by the progenity of one pair of moths. This destruction, therefore, can annihilate the pollination advance to the plant. To the moth the relationship is always profitable. Adults feed from the flowers and the larvae eat the seeds. The relationship, therefore, is a form of predation by the moth. It differs much from the balanced relationship of the Yucca moth with Yucca flowers. *M. album* survives because each plant lives for several years and because there are often some capsules overlooked by the caterpillars. Other moths species also provide pollination.

From an evolutionary point of view, the relationship between *Hadena* and Caryophyllaceae is supposedly an old one. Most *Hadena* species share the same family of food plants and eat the same flower parts. This indicates that the relationship existed for their common ancestor.

A second argument could be the high efficiency with which *Hadena* utilises *M. album*: A) the moth prepares food for the caterpillars by pollination. B) oviposition is in a well defined place on the plants, only inside the flowers, and C) the moth distinguishes between staminate and pistillate flowers with a high degree of precision.

It is tempting to regard this predatory relationship as an intermediate fase in the evolution of a relationship with mutual benefit as hypothesises by OLBERG (1951). However, there is no evidence that evolution will continue in this direction, or that the relationship can be perfected at this moment. All aspects point to a high perfection in the utilisation of *M. album*. For further evolution to a less one-sided relationship, the predator has to be made to restrict damage to its host. As *M. album* is a common weed there has obviously never been the danger of extinction caused by its predator. *M. album* occurs mainly in cultivated land, and along roadsides. Changes in cultivation methods are a great threat to its abundance. *H. bicruris* is only one of the pollinators of *M. album*. The damage from *Hadena* might be compensated by pollination by other species. On the other hand *Hadena* can feed from other Caryophyllaceae, and does so. Therefore, there is no clear selective advantage for *H. bicruris* to restrict the damage to *M. album*. Evolution in this direction is uncertain. The only line of evolution that might be expected is for *M. album* to evolve pistillate and staminate flowers which could easily be confused by the moth, or to evolve a reduction in the nutritive value of the ovules for the larvae. One might ask whether other members of the subfamily Silenoidae in fact did escape in such a way from the attack by *Hadena* species. In this way the predator might have been one of the selective forces acting upon the evolution of the Caryophyllaceae.

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REFERENCES

BLASCHE, P., Raupenkalender für das Mitteleuropäische Faunengebiet. Alfred Kernen Verlag, Stuttgart, (1955).

BRANTJES, N. B. M., Sphingophilous flowers, function of their scent. In: Brantjes, N. B. M., Linskens, H. F. (ed.), Pollination and Dispersal. Publ. Dept. Botany, Nijmegen, 27–46, (1973).

————, De tong van een vlinder. Vakblad voor Biologen, 53 108, (1973).

EIDMANN, H. and F. KUHLHORN, Lehrbuch der Entomologie. Paul Parey Verlag, Hamburg, (1970).

IMMS, A. D., A general textbook of entomology. Methuen & Co., London, 9th ed., (1964).

KISLEV, M. E., Z. KRAVIZ and J. LORCH, A study of hawkmoth pollination by a palynological analysis of the proboscis. Israel Journal of Botany 21, 57–75, (1972).

KNOLL, F., Lichtsinn und Blumenbesuch des Falters von *Macroglossum stellatarum*. Abh. Zool. Bot. Ges., Wien, 12, (2) 123–376, (1922).

KOCH, M., Wir bestimmen Schmetterlinge. III, Eulen Deutschlands. Neuman Verlag, Radebeul, (1958).

LEMPKE, B. J., Catalogus der Nederlandse macrolepidoptera, suppl. 10. Tijdschrift voor de entomologie, 107, 49–136, (1964).

MARSDEN-JONES, E. M. and W. B. TURRILL, The bladder campions. Ray Society, London, (1957).

MULCAHY, D. L., The optimal sexratio in *Silene alba*. Heredity 22, 411–423, (1967).

————, The significance of delayed pistillate anthesis in *Silene alba*. Bull. Torrey Bot. Club 95, 135–139, (1968).

OLBERG, G., Blute und Insekt. Acad. Verl. Gesl. Geest & Portig K.G., Leipzig, (1951).

SCHREMMER, F., Sinnesphysiologie und Blumenbesuch des Falters von Plusia gamma L. Zool. Jb. Syst. 74, 375–435, (1941).

SEPPANEN, E. J., The food-plants of the larvae of the Macro-lepidoptera of Finland. Anim. Fenn. 14, 1–179, (1970).

SIEGEL, S., Nonparametric statistics for the behavioural sciences. Mc. Craw-Hill Book Comp. Inc., New York, (1956).

SOUTHWOOD, T. R. E., The insect/plant relationship- an evolutionary perspective, (1972). In: Emden, H. F. van (ed.), Insect/plant relationships. Blackwell scientific publications, Oxford, p. 3–32, (1973).

SPULER, Schmetterlinge Europa's, IV. Die Raupen. Schweizerbart'sche Verlags Buchh., Stuttgart, (1910).

WHITE, F. B., The influence of insect agency on the distribution of plants. The Journal of Botany, british and foreign, London, New series, 2, 11–13, (1873).

WIGGLESWORTH, V. B., The principles of insect physiology. Methuen & Co., London, (1972).

WILDE, O., Die Pflanzen und Raupen Deutschlands. Versuch einer lepidopterologischen Botanik. II. Systematische Beschreibung der Raupen unter Angabe ihrer Lebensweise und Entwicklungszeiten. E. S. Mittler & Sohn, Berlin, (1861).

Oecologia (Berl.) 24, 1–6 (1976)

Oecologia

Prevention of Superparasitation of *Melandrium* Flowers (Caryophyllaceae) by *Hadena* (Lepidoptera)

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Summary. The preferences of *Hadena bicruris* for oviposition into pistillate plants of *Melandrium album* were observed in the Botanical Garden of the University of Nijmegen. Statistical analysis showed that each night most eggs are deposited on certain plants. Second-day flowers receive less eggs than first-day flowers. Flowers containing an egg have a lowered propability of receiving a second one. They have a "mark", which functions only one night. This prevention of superparasitism, unique for Lepidoptera, is of survival value for the moth species.

Introduction

Hadena bicruris Hufn. (Noctuidae, Lepidoptera) drinks nectar from flowers of *Melandrium album* Garcke (Caryophyllaceae) and pollinates them. After drinking, the females often deposit an egg into the pistillate flowers only. This behaviour and the way the moth distinguishes between pistillate and staminate flowers has been analysed experimentally in detail elsewhere (Brantjes, 1976).

During those experiments it was observed that a tendency of the moths to skip visited flowers seemed to exist: the moths approached these flowers, but often did not land. Parasites, perceiving previous parasitation of their host and consequently not depositing a second egg, are known for Hymenoptera (Lenteren, 1972; Lenteren et al., 1975) and for Coleoptera (Mitchell, 1975; Oshima et al., 1973).

The present study deals with additional features of the pistillate flowers, viz. those possibly regulating selection for oviposition by the moth, especially in connection with previous visits to the flowers.

As compared to those in the field, under laboratory conditions the selectivity of the female moths turned out to be much less pronounced. Therefore, only under field conditions minor preferences for ovipositing substrates can be detected.

Materials and Methods

In the Botanical Garden of the University of Nijmegen 5 pistillate plants were grown, standing in a row along a path about 30 m apart

Unless stated otherwise, all flowers were picked off each day and checked for eggs Therefore, the flowers were exposed for only one night

During observations on the influence of age of the flower, a thread (each day of different colour) was tied around the pedicel of flowerbuds at the eve of opening, in order to be sure about the age of the flowers after several nights Also during observations, the flowerbuds of all staminate plants of *M album* in the garden were removed to prevent pollination and consequent wilting of the pistillate flowers

Differences between observed and expected numbers of eggs have been tested for significance with chi-square Only expectance values greater than 5 0 were included in the calculations

Over the whole observation period (15 7–14 8 1975) weather conditions remained constant and there was no rain At 1 m above the soil, between the vegetation, wind velocity never exceeded 3 m per second, and was mostly much less

Air temperature at 20 cm above the ground followed a fixed pattern each day At approximately 6.00 a m a continuous rise started from 18° C up to the day maximum (35–42° C) at about 1 00 p m At 5 00 p m temperature began to decrease After 6 00 p m the decrease was very fast until at 10 00 p m the temperature reached 21° C During the night there was a slow decrease to 18° C.

Air relative humidity followed an inverse pattern with, at daytime, minima of 20 to 15%, and maxima during the night varying from 60 to 95%

Observations

Eggs deposited in first-day flowers are collected in order to see whether some plants are preferred to others (Table 1). In case of equal attractiveness for oviposition, the expectance of each plant would be proportional to its number of flowers. However, the observed distribution of the eggs over the plants often differs significantly from the expected one. Therefore, on each night eggs are not deposited at random over the garden, but oviposition is concentrated on certain plants. As each of these receives more eggs than expected on one night or another, it is unlikely that systematic factors are involved.

In case all flowers on a single plant are equally attractive for oviposition, the distribution of the eggs over the flowers will be binominal (Brantjes, 1976). The expected number of flowers with 0, 1, 2 or 3 eggs can be calculated with the formulae given in Figure 1. If on a plant some flowers are more attractive than others, these will receive more eggs. Consequently we will observe more flowers with 2 or 3 eggs than expected.

Countings of number of eggs per flower made on 5 successive nights are given in Table 2. Because of the observed tendency to concentrate eggs on some plants, the expectance is calculated for each single plant and added to obtain the total expected frequency distribution for each night.

Every night the observed distribution deviates significantly from the expected one. However, there are always *less* flowers with 2 or 3 eggs than expected.

This is the reversal of the situation of varying attractiveness as was shown before. The eggs are distributed over the flowers more evenly than would be the case after random deposition.

Table 1. Distribution over the plants of the eggs deposited during one night

Date		Plant number						significance of o–e
		1	2	3	4	5	Total	*P*
30 7–31 7	f	10	133	8	0	37	188	0 1–0 05
	o	12	39	0		17	68	
	e	3 6	48*	2 8		13 3*		
31 7–1 8	f	18	182	9	0	9	218	0 1–0 05
	o	11	37	9		1	58	
	e	4 8	48 4	2 4		2 4		
1 8–2 8	f	11	173	5	0	23	213	0 3–0 2
	o	10	63	3		6	82	
	e	4 2	67*	1 9		8 9*		
10 8–11 8	f	5	5	20	6	10	46	0 01–0 001
	o	6	5	9	8	19	47	
	e	5 1*	5 1*	20 4*	6 1*	10 2*		
11 8–12 8	f	3	6	25	2	9	45	0 1–0 05
	o	5	9	15	1	10	40	
	e	2 6	5 3*	22 2*	1 8	8 0*		
12 8–13 8	f	7	8	15	4	18	52	0 001
	o	4	33	7	0	16	60	
	e	8 1*	9 3*	17 3*	4 6	20 8*		
13 8–14 8	f	13	17	32	9	23	94	
	o	16	7	3	5	7	38	
	e	5 3*	6 8*	12 9*	3 6	9 3*		

f = number of flowers on the plant, o = observed number of eggs on the plant, e = expected number of eggs on the plant

Calculation of significance, see "Material and Methods"
Values included in calculation are marked with *
P = probability H_0 (no systematic deviation) remains true

Fig. 1. Formulae for calculation of the expected distribution of eggs over the flowers of one plant

a = total number of flowers
b = total number of eggs
$c(\ )$ = expectance of flowers with eggs

$c(0) = a\ ((a\ 1)/a)^b$
$c(1) = b\ ((a\text{-}1)/a)^{(b\ 1)}$
$c(2) = (b(b\text{-}1)/2a)\ ((a\text{-}1)/a)^{(b\ 2)}$
$c(3) = (b(b\ 1)(b\ 2)/6a^2)\ ((a\text{-}1)/a)^{(b\text{-}3)}$

The formulae are derived from the binomial distribution

The conclusion is that the moths select the flowers to oviposit in, and that flowers containing an egg have a lowered probability of receiving a second, not to mention a third one

Brantjes (1976) has shown that the decision to oviposit or not is made after approach to the flower Obviously the moth perceives the presence of the egg

Table 2. Frequency distribution of the eggs in the flowers

Date		Number of eggs per flower				Significance of difference o–e
		0	1	2	3	*P*
30 7–31 7	o	115	62	3	0	0 01–0 001**
	e	125 1	44 0	8 7	1 58	
31 7–1 8.	o	155	53	3	0	0 05–0 02*
	e	156 0	40	6 6	0 8	
1. 8–2 8.	o	136	70	7	0	0 05–0 02*
	e	146 4	55 4	11 3	1 8	
2 8–3. 8	o	119	55	12	0	0 5–0 3
	e	121 5	51	16 6	1 5	
4 8–5 8	o	68	57	4	0	0 01–0 001**
	e	77 8	39 5	9 8	1 6	
Total	o	593	297	29	0	0 001–***
	e	626 8	230	53	7 3	

o=observed frequency, e=expected frequency, calculation see text, *P*=probability H_0 (no systematic deviation) remains true

* Significance 0.05, ** 0 01, *** 0 001

inside the flower or other markings left behind by moths ovipositing into the flowers previously. This cue by which the moth perceives the presence or absence of an egg will be called simply: "mark".

Only because the total number of eggs deposited was high, related to the number of flowers, the functioning of a mark could be demonstrated by statistic approach. But also on other (15) nights of observation the mark was functioning, as was clear from the absence of flowers with 2 eggs on these nights. However, the total number of eggs was insufficient for calculation of significance.

Pistillate flowers remain functional for many days until pollination takes place. Therefore, in the field pistillate flowers of different ages are available for egg deposition. The number of eggs in flowers of one day and in flowers of two days old on the same plants, were compared in order to check the influence of age (Table 3). Eggs deposited on the night preceeding the night of observation can easily be distinguished by their contents, which after one day darkens from white to brown.

The second day flowers obtained a significantly lower number of eggs as compared to the first day flowers. Therefore, attractiveness for oviposition decreased with age of the flower.

The mark might result from metabolic processes of the flower after reception of an egg. If this hypothesis is valid, the mark would be more pronounced in flowers with an egg of the preceeding night. To see whether the mark lasts in the second night, the two nights old flowers are sorted out into two categories: a) those with an egg from the night before, and b) those without. The distribution of eggs of the second night (Table 4) compares very well with the distribution expected in the case the old egg does not influence the deposition of new

Table 3. Influence of age of the flower on egg deposition

Date		Number of eggs deposite during one night in		Significance e–o
		first night flowers	second night flowers	*P*
11 8 12. 8	f	24	20	0 01–0 001
	o	14	1	
	e	8.2	6 8	
13. 8 –14 8.	f	44	57	0 03–0 02
	o	27	18	
	e	19.3	25.7	

f, e, o, *P* as in Table 1

Table 4. Influence of old eggs on egg deposition

	Second night flowers selected by the moths to oviposit in	
	a) with an egg from the night before	b) without eggs
Observed	7	6
Expected	6 1	6 9

ones. Therefore, the mark seems not to last until the second night. Thus, metabolic processes of the flower do not seem to be involved in the recognition of previous infection.

Discussion

The advantage of the mark to the moth is clear from the following observations. Each caterplillar of *H. bicruris* consumes more than 5 seedcapsules of *M. album*. Therefore, two caterpillars feeding on the same capsule are competitive. In breeding experiments it has been observed that first instar larvae are cannibalistic. Older ones are not, possibly because they can avoid a too close contact. Two or more eggs put into one flower, therefore, result in loss of all but one. Economy of eggs was also met with as motive for the distinction made by the moths between pistillate and staminate flowers (Brantjes, 1976).

The existence of a capacity for discrimination between parasitised and unparasitised plants has possible potentialities with regard to pest management.

The flowertype (staminate versus pistillate) was found to be decisive in regulating selection of substratum for egg deposition by *H. bicruris* (Brantjes, 1976).

To this factor now is added the age of the flower: the second night flowers being less attractive than the first night flowers. Because some eggs were deposited into second night pistillate flowers, and none into staminate flowers, the influence of age is small as compared to that of the flowertype. Also the influence of the mark is not as absolute as that of the flowertype.

Differences between individual pistillate plants, however, did not seem to influence systematically the selection for the oviposition.

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References

Brantjes, N B M Riddles around the pollination of *Melandrium album* Garcke (Caryophyllaceae) during the oviposition by *Hadena bicruris* Hufn (Noctuidae, Lepidoptera) Proc kon ned Akad Wet , Series C, **79**, 1–12 (1976)

van Lenteren, J C Contact-chemoreceptors on the ovipositor of *Pseudocoïla bochei* Weld (Cynipidae) Neth J Zool **22**, 347–350 (1972)

van Lenteren, J C , Bakker, K Discrimination between parasitised and unparasitised hosts in the parasitic wasp *Pseudocoïla bochei* a matter of learning Nature (Lond) **254**, 417–419 (1975)

Mitchell, R The evolution of oviposition tactics in the bean weevil *Callosobruchus maculatus* (F) Ecology **56**, 696 (1975)

Oshima, K , Honda, H , Yamamoto, I Isolation of an oviposition marker from the Azuki bean weevil, *Callosobruchus chinensis* (L) Agric Biol Chem **37**, 2679–2680 (1973)

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OVERZICHT VAN DE ROL VAN DE BLOEMGEUR BIJ DE BLOEMBESTUIVING

Om de rol van de bloemgeur nauwkeurig te kunnen bespreken wordt het gedrag van de vlinders in 4 fasen onderscheiden die bij alle nachtvlinders aanwezige fasen blijken te zijn:
1) activatie van het voedselzoekgedrag, 2) oriëntatie naar de bloem, 3) bloembezoek en landing, 4) vinden van de bloemopening. Daarbij komt voor wijfjes van *Hadena* spp. soms een 5e fase: het leggen van een ei. De rol van de geur zal hier voor iedere fase afzonderlijk worden bekeken. Bovendien zal over het vermogen tot leren van de vlinders worden besproken. Nagegaan zal ook worden welke consequenties de reacties van de vlinders op geur hebben voor de bloembestuiving.

Activatie van voedselzoekgedrag

Gewoonlijk, in de experimentenkooi, vliegen de pijlstaarten bovenin traag in het rond (Brantjes 1973). In de kooi staande voorwerpen worden waargenomen, want de vlinders ontwijken deze bij het langs vliegen. Vaak wordt naar lichte plekken in de kooiwand en in het dak gevlogen. Daar stoten de vlinders dan herhaaldelijk tegen, als om te ontsnappen. Zodra een daarvoor geschikte (zie onder) geur in de kooi wordt gebracht gaan de vlinders op en neer vliegen en zigzaggen rond in de kooi: de vlinders vertonen nu de karakteristieke "zoekvlucht". Lichte of gekleurde voorwerpen, die eerst ontweken werden, worden nu benaderd. Ervoor gekomen strekken de vlinders de tong uit en betasten het voorwerp ermee. Onder invloed van de geur veranderen dus de reacties op (geurloze) voorwerpen. Deze zoekvlucht en die reacties op de voorwerpen leiden gewoonlijk tot bloembezoek en zijn dus elementen van het voedselzoekgedrag, de "feeding drive" (Brantjes 1973). De perceptie van geur activeert dit gedrag. Geur kan dus een "sign-stimulus", in de betekenis van Tinbergen (1955), zijn voor de activatie van het voedselzoekgedrag.

Eenmaal geactiveerd blijft de vlinder langere tijd doorgaan met het bloembezoek, tot verzadiging of tot een ander gedragspatroon geactiveerd wordt.

Onder gunstige omstandigheden kunnen zittende pijlstaarten ook door inbreng van geur tot vliegen gebracht worden.

Niet alle geuren blijken effectief (Brantjes 1973). Opwekken van het voedselzoekgedrag is bij *Manduca sexta* met *Petunia hybrida* bloemengeur en met stoffen als Isoamyl-benzoaat, Isoamylsalicylaat, n-Amylsalicyaat gelukt. *Deilephila elpenor* daarentegen is niet door deze stof-

fen activeerbaar, maar wel door de bloemgeur van *Lonicera periclimenum*. De reactie lijkt dus specifiek te zijn bij deze pijlstaarten.

Het voedselzoekgedrag wordt niet uitsluitend door geur geactiveerd. Het kan spontaan optreden bij de start van het vliegen in de avond, of bij verlaging der prikkeldrempel na langere tijd geen voedsel gehad te hebben. Zo wordt het begrijpelijk dat Knoll (1922) tot de overbodigheid van geur voor het bloembezoek door *Macroglossum stellatarum* besloot; hoewel hij de karakteristieke plotseling optredende "Nahrungstrieb" wel noemt zonder echter over de activatie ervan te spreken, betroffen zijn waarnemingen steeds hongerige dieren.

Ook bij noctuidae werkt geur als activator van bloembezoek gedrag. Het vliegbeeld van de vlinders die bovenin de kooi rondvliegen, verandert direct na inbreng van bloemgeur in een karakteristieke zoekvlucht, die lager in de kooi en met veel wendingen wordt uitgevoerd. Dit is beschreven voor *Autographa gamma* (Schremmer 1941) en *Hadena bicruris* (Brantjes 1976 b). Bij deze vlinders kunnen geuren van meerdere bloemen, zoals bijvoorbeeld *Melandrium album, Saponaria officinalis* en *Phlox paniculata,* zoekvluchten oproepen. De activatie is dus, in tegenstelling tot bij de sphingidae, weinig geur-specifiek.

Wij concluderen uit het bovenstaande dat bij de beide onderzochte vlindergroepen de geur de sign-stimulus kan zijn voor het voedselzoekgedrag, dat herkenbaar is aan de zoekvlucht.
De pijlstaarten reageerden meer specifiek, terwijl uilen op meerdere bloemgeuren en niet specifiek reageerden.

Oriëntatie naar de bloem

Over grote afstanden, zolang de bloemen nog niet zichtbaar zijn, kan gerichte oriëntatie naar bloemen alleen via de geur geschieden. Er zijn twee mechanismen mogelijk:
a) oriëntatie door middel van gradiënten in geurconcentratie, de hierna te bespreken osmotropotaxis, en b) de door geur opgeroepen positieve anemotaxis. Dat is: een tegen de wind in vliegen zolang de geur, onafhankelijk van concentratie verschillen, wordt waargenomen.

Experimentele onderzoekingen die op een dergelijke positieve anemotaxis bij pijlstaarten en uilen bij bloembezoek wijzen zijn er niet. Wel zijn er enkele veldwaarnemingen. Tinbergen (1953) zag *Hyloicus pinastri* tegen de wind in naar verborgen *Lonicera* bloemen vliegen. Ook Lederer (1959)

zag veel pijlstaarten tegen de wind in bloemen benaderen. In het veld vliegen ook uilen meest tegen de wind in naar bloemen. Echter deze observaties van tegen de wind in vliegen vormen onvoldoende bewijs voor het optreden van positieve anemotaxis. Het tegen de wind in vliegen zou ook functioneel kunnen zijn bij het optisch localiseren van bloemen en het landen erop.

Bij afwezigheid van luchtstromingen kunnen vlinders voor hen onzichtbare bloemen vinden door middel van osmotropotaxie. Dat wil zeggen: door tegelijkertijd waarnemen van geurconcentratie-onderscheid tussen twee verschillende punten, zoals b.v. de uiteinden van de antenne (Martin 1964). Deze osmotropotactische oriëntatie is bij vlinders herkenbaar aan de karakteristieke wijze van benaderen van de geurbron: met gespreid gehouden antennes, langzaam in lussen vliegend. Dit is geobserveerd bij *Autographa gamma* (Schremmer 1941), *Cucullia umbratica* (Brantjes 1976 a) en *Hadena bicruris* (Brantjes 1976 b).

Tijdens de experimenten zijn voor ieder van de vlinders zeer veel bloemengeuren attractief gebleken.

In de experimenten waarbij bloemextracten op filtreerpapier de geurbron vormen (Brantjes 1976 b), worden door ten opzichte van bloemen of geurbronnen nog onervaren *H. bicruris* vlinders de stroken filtreerpapier met de sterkste geur het meest bezocht. Dit wijst er op dat deze vlinders zich naar de hoogste geurconcentratie richten.

In een afgesloten kas in de Botanische tuin blijken *H. bicruris*, *A. gamma* en *C. umbratica* op deze wijze slechts de geurbron te naderen als ze daar niet verder dan enkele meters van verwijderd zijn. Wanneer de afstand groter is blijven de vlinders ongericht of in zoekvlucht vliegen zonder de geurbron te bereiken. Alleen daar waar de concentratie-verschillen, veroorzaakt door het concentratieverval van de geurbron af, waarneembaar groot zijn, is osmotropotaxie mogelijk.

In het veld zal de mate waarin door de diverse soorten Noctuidae van het gezicht of van de reuk gebruik gemaakt wordt voor het benaderen van de bloemen verschillen.
De duidelijk verschillende manier waarop de vlinders in het veld de bloemen benaderen wijst hier al op. Zo vliegt *C. umbratica* snel rechtuit naar de bloem, wat wijst op visuele oriëntatie. *A. gamma* en *H. bicruris* daarentegen vliegen meest rustig, een beetje slingerend rond en benaderen de bloemen langzaam van onderen af. Dit vliegbeeld is karakteristiek bij geuroriëntatie. Soms benadert *H. bicruris* ook geurloze witte bloemmodellen. Oriëntatie op het gezicht is bij deze soort dus ook mogelijk.

De geuroriëntering is volgens Schremmer (1941) de enige wijze waarop pas uit de pop gekomen *A. gamma* uilen hun eerste bloemen vinden. Visueel benaderen de vlinders de bloemen pas na enkele bloembezoeken, als een associatie tussen het beeld van de bloem en het voedsel gevormd is.

Ook pijlstaarten kunnen zonder wind een geurbron localiseren: in de experimenteerkooi is *Deilephila elpenor* zigzaggend naar de opening van een doosje, waarin *Lonicera periclimenum* bloemen verborgen waren, gevlogen en daar met uitgestrekte tong geland (Brantjes 1973). Maar geur is voor oriëntering niet onmisbaar. Want, zodra het voedselzoekgedrag door geur eenmaal is opgeroepen, benaderen zowel ervaren als onervaren pijlstaarten, in afwezigheid van geurende bloemen, wel geurloze gekleurde voorwerpen en proberen te drinken. Gelijke waarnemingen die Knoll (1922, 1925) deed met *Hyles livornica* en *Macroglossum stellatarum* brachten hem tot de foutieve conclusie dat geur voor pijlstaarten geen functie heeft bij bloembezoek.

Dat *D. elpenor* ook werkelijk geur als oriëntatiemiddel benut blijkt uit observaties dat tussen bloembezoeken door wel eens tussen de bloemen geplaatste geurbronnen van onzichtbare bloemen benaderd worden (Brantjes 1973).

Uit het gedrag van de vlinders valt op te maken dat bij de eerste bloembezoeken naast mogelijke visuele signalen ook geur als oriëntatiemiddel benut wordt. Bij hun eerste bloembezoeken namelijk benaderen zowel *D. elpenor* als *M. sexta* de bloemen langzaam en van beneden af. Bij latere bezoeken vliegen deze vlinders aan op gelijke hoogte van de bloem of van boven af. Dit wijst meer op visuele oriëntatie.

Deze visuele oriëntatie heeft als voordeel voor de vlinder dat het direct en snel gaat. We kunnen dan ook verwachten dat in het veld, als de vlinders vele bloemen na elkaar afwerken, de visuele oriëntatie domineert.

Samenvattend kunnen we dus stellen dat ook bij het zoeken van bloemen de bloemgeur een rol speelt voor nachtvlinders. Benaderen door via geur opgeroepen positieve anemotaxis van grote afstanden is niet uit te sluiten. In de nabijheid van de bloemen kan *D. elpenor*, ook onervaren, zowel osmotropotactische als visuele oriëntatie benutten. Terwijl het gedrag van *M. sexta* en *D. elpenor* er op wijst dat bij het begin van het bloembezoek geuroriëntatie overheerst en later wordt vervangen door visuele oriëntatie. Ook Noctuidae kunnen van nabij door geurconcentratie verschillen de bloemen vinden. Met name onervaren vlinders doen dit bij

hun eerste bloembezoeken. Sommige blijven dit in combinatie met visuele oriëntatie ook bij verdere bloembezoeken doen.

Bloembezoek en landing

In de experimenteerkooi vliegt *H. bicruris* soms in de richting van witte, geurloze voorwerpen, zoals bijvoorbeeld strookjes filtreerpapier. Tot landen komt het echter hoogst zelden. Na het voorwerp tot op enkele centimeters benaderd te hebben keren de vlinders zich er van af. Maar wanneer hetzelfde voorwerp met een extract van bloembladeren van geur is voorzien, landen de vlinders wel (Brantjes 1976 b). Door het ontbreken van geur wordt blijkbaar niet tot landen overgegaan. Geur blijft, ook voor ervaren nachtuilen, essentieel voor de beslissing te landen en het ontrollen der tong.

Dat geur alleen reeds voldoende is voor het ontrollen der tong blijkt uit het feit dat de vlinders ook bij de achter gaas verborgen bloemen landen, waarna de tong ontrold en door het gaas gestoken wordt.

Na door geur geactiveerd te zijn, vliegen pijlstaarten daarentegen met gestrekte tong ook geurloze voorwerpen aan. De beslissing om tot bezoek aan een concreet voorwerp over te gaan kan hier dus wel zonder geur aan dit voorwerp worden genomen.

Bij *D. elpenor* evenwel kan geur van verborgen bloemen wel het ontrollen van de tong induceren, zoals hierboven beschreven is.

Het belang van bloemengeur bij de beslissing tot landen is dus voor Sphingidae en Noctuidae verschillend.

Vinden van de bloemopening

Als vierde fase in het bloembezoek beschouwen wij het vinden van de bloemopening en het insteken van de tong. Pijlstaarten richten de tong op visuele honingmerken (Knoll 1925). Of ook geurmerken werkzaam zijn voor pijlstaarten is niet bekend. Onmisbaar zijn deze kennelijk niet, immers ook bij achter glas opgestelde bloemen richten pijlstaarten de tong altijd correct op de bloemopening.

Dat bij een typische nachtvlinderbloem als *Platanthera bifolia* de geur slechts op enkele plaatsen van de bloem wordt geproduceerd (Vogel 1962), zou op geurmerken voor pijlstaarten kunnen duiden. Deze plant wordt

echter niet uitsluitend door pijlstaarten bestoven (Heinsius 1890, Darwin 1877). Een richtende invloed van gelocaliseerde geuremissie kan daarom ook functioneel zijn voor andere vlindergroepen.

Een aanwijzing dat geurhoningmerken voor Noctuidae wel van belang kunnen zijn ligt al in het feit dat deze na het landen het gehele bloemoppervlak met antennes aftasten, terwijl pijlstaarten de antennes meestal stijf, schuin naar voren gespreid houden. Het blijkt in experimenten dat voor *C. umbratica* de bloemkroon van *Melandrium album* een richtende werking heeft bij het insteken van de tong (Brantjes 1976 a). Ook als de vlinder onder de bloem hangt zodat de bijkroon niet gezien kan worden blijft de werking behouden.

Dat geurmerken slechts voor Noctuidae als honingmerk functioneren kan gedragsmatig bepaald zijn.
Noctuidae immers landen op de bloemen waarna ze met de antennes de bloem kunnen aftasten. Sphingidae daarentegen zweven voor de bloem en veroorzaken daarbij een grote luchtturbulentie, waardoor de geur mogelijk te zeer verwaait om localisatie van geur-verschillen op de bloem mogelijk te maken.

De ovipositie

Hadena bicruris kiest in het veld voor ovipositie alleen de stamper- (vrouwelijke-) bloemen van *Melandrium album*, een tweehuizige plant. Het eileggedrag waarbij de eieren met grote precisie diep in de bloem worden gelegd, is door ons beschreven (Brantjes 1976 b). Beide geslachten van de vlinders bezoeken voor drinken alleen stamper- en meeldraad-bloemen door elkaar. Ze bestuiven daardoor effectief. Voor de ovipositie in een bloem drinken de vlinders er altijd uit. De substraatkeuze voor ovipositie en het drinkgedrag lijken in één ondeelbare gedragsketen verbonden. Dit blijkt ook als het drinken wordt geblokkeerd door verzadiging van de vlinders: de eieren worden dan niet meer in de aanwezige bloemen, maar erbuiten gelegd. Bloemen die de eerste nacht bloeien, hebben een grotere kans op een ei dan bloemen van de tweede nacht. Bij afwezigheid van stamperbloemen worden ook eieren in meeldraadbloemen gedeponeerd. Dus beide bloemtypen bevatten een ovipositie-richtende factor, maar die van de stamperbloemen is blijkbaar sterker.

Door verschillende combinaties van bloemdelen in kunstmatige bloemen te plaatsen en na te gaan in welke mate de vlinders eieren legden in deze kunstbloemen is aangetoond dat alleen de kroonbladeren een richtende invloed hebben (Brantjes 1976 c). Hiervan dragen de plaat en de

bijkroon beide aan het ovipositie-richtend effect bij. De meeste eieren worden gelegd in kunstbloemen met het grootste aantal kroonbladeren. De selectie van de ovipositieplaats is dus kwantitatief bepaald. Dit blijkt ook als wij het aantal kroonbladeren en het bloemtype (stamper- of meeldraadbloem) gelijktijdig variëren: het aantal kroonbladeren is dan belangrijker dan het bloemtype.

Omdat de stamperbloemen voor ovipositie geprefereerd worden en omdat de preferentie kwantitatief bepaald is, moet men aannemen dat in de kroonbladeren van de stamperbloemen de richtende factor in grotere mate aanwezig is dan in de kroonbladeren van de meeldraadbloemen. Om na te gaan wat de aard van de richtende factor is, zijn in het donker bloemen achter gaas aangeboden, zodat gezicht en direct contact uitgesloten zijn. De eieren blijken dan op het gaas vlak bij de bloemen te worden gelegd. Geur blijkt dus reeds voldoende.

Uit het gedrag van de vlinders kan men op maken dat het moment waarop het bloemtype-perceptie wordt waargenomen vlakbij het moment van de landing ligt en wel vóór het drinken (Brantjes 1976 c). Vermoedelijk wordt de beslissing tot ovipositie gelijktijdig met de beslissing om te landen en te drinken genomen.

Zoals gezegd zijn drinken en ovipositie in één gedragsketen verbonden. Omdat, zoals hierna besproken zal worden *H. bicruris* vermogen tot leren toont bij het bloembezoek bestaat de mogelijkheid dat ook de ovipositie-preferenties door ervaring beïnvloedbaar zijn, speciaal door bij het drinken gevormde associaties. Immers vlinders die uitsluitend uit *Phlox* bloemen gevoed zijn leggen ook eieren in deze bloemen, mits er geen *M. album* bloemen aanwezig zijn. In aanwezigheid van *M. album* bloemen echter, ook wanneer deze geen nectar bevatten, wordt de ovipositie hierop gericht, hoewel de associatie met *Phlox* als nectarbloem blijft bestaan. De preferentie voor de bloemsoort bij de ovipositie blijkt dus slechts weinig door ervaring modificeerbaar. Ook zonder er uit gedronken te hebben heeft de vlinder een aangeboren voorkeur voor ovipositie in *M. album* bloemen. Hoewel drinken en ovipositie in tijd en plaats verbonden zijn worden beide toch door onafhankelijke factoren gestuurd. Immers voor drinken kunnen veel bloemen-geuren richtend zijn en door ervaring zijn de voorkeuren modificeerbaar. Daarentegen wordt ovipositie slechts op één type van één bloemsoort gericht en slechts in afwezigheid daarvan beïnvloedt ervaring de ovipositiepreferenties.

Behalve dat *H. bicruris* wijfjes het verschil tussen de geur van mannelijke en vrouwelijke bloemen waarnemen, bemerken zij ook of er reeds dezelfde nacht een ei in de bloem is afgezet (Brantjes 1976 d). Het ei zit diep in de bloem zodat zichtbaarheid ervan vrijwel is uitgesloten. Daar de beslissing tot ovipositie reeds bij het landen, op basis van geur, wordt genomen, lijkt een geurmerk, door de eierleggende voorganger aan de bloem afgegeven, waarschijnlijk.

H. bicruris voorkomt zo dubbele infectie, superparasitering (niet te verwarren met hyperparasitering, dat wil zeggen infectie van een parasiet), en daarmee eiverspilling. De enige bekende analogie bij vlinders is bij *Heliconius* beschreven Uit gedragsobservaties en uit het bestaan van door de plant gevormde ei-imitaties concludeert Gilbert (1975) dat *Heliconius* voor ovipositie de plant ook eerst (hier visueel) controleert op de aanwezigheid van eerder gelegde eieren.

Vermogen tot leren bij drinken

Manduca sexta en *D. elpenor* vlinders, die nog nooit gedronken hebben, zodat nog geen associatie kleur-voedsel bestaat, richten zich op geurloze bloemmodellen. Deze reactie op kleur is dus aangeboren. Daarnaast kunnen pijlstaarten leren. Knoll (1925) laat zien hoe zich na enkele bloembezoeken reeds associaties met kleuren kunnen vormen. Het leren is hierbij een modificeren van een aangeboren voorkeur voor gekleurde voorwerpen bij het zoeken van nectar. Behalve bij de beslissing om tot het bezoeken van een bloem over te gaan is de, voor Sphingidae ook 's nachts waarneembare, kleur dus ook van belang bij de selectie van de bloem.

Wat betreft de Noctuidae: Schremmer (1941) laat zien dat *A. gamma* associaties kan vormen tussen de geur en de kleur van bloemen enerzijds en de nectar anderzijds. Associaties tussen geur en nectar blijken sterker te zijn dan associaties tussen kleur en nectar. Wegens de geringe afstand waarover bloemen, speciaal in meng-populaties, aan hun geur herkend zullen kunnen worden, beïnvloedt in het veld de kleur mogelijk de keuze van de te benaderen voorwerpen. Bij de beslissing om te landen zal de sterkere associatie met de geur waarschijnlijk de doorslag geven.

De snelheid waarmee *H. bicruris* leert wordt geïllustreerd door experimenten waarin op filtreerpapier extracten van *M. album* bloembladeren worden aangeboden. Als er op dit filtreerpapiertje geen suikerwater als remplacant van nectar is landen vlinders voor de eerste keer wel snel op de papiertjes en zoeken het oppervlak langdurig met de tong af. Een

tweede papiertje bezoeken ze daarna nog slechts zelden, een derde nooit meer.

Specificiteit en trouw der bloembezoekers

De geurspecificiteit bij het oproepen van bloembezoekgedrag bij Sphingidae kan leiden tot specificiteit in bloembezoek. Daar geactiveerde pijlstaarten echter alle gekleurde voorwerpen proberen, zullen ook andere bloemsoorten in de omgeving wel bezocht worden. Uit veldobservaties (Kislev 1972, Baker 1961, Gregory 1963) blijkt dan ook dat Sphingidae weinig trouwe bezoekers zijn. Trouw kan zich echter wel ontwikkelen op basis van kleuren, zoals Knoll (1925) laat zien. De bloemen moeten dan wel voldoende nectar leveren en herkenbaar zijn (Gregory 1963).

De onderzochte Noctuidae daarentegen worden niet door specifieke geuren geactiveerd. Constantie, gebaseerd op kleur of geur, ontwikkelt zich wel, maar in het veld blijkt de bloemtrouw van Noctuidae laag te zijn.

Zowel voor Sphingidae als voor Noctuidae geldt dat de mate van bloemtrouw meer van de omstandigheden in het veld afhangt dan van de capaciteiten der dieren.

REFERENTIES

Baker, H.G., 1961. The adaptation of flowering plants to nocturnal and crepuscular pollinators. Quart. Rev. Biol. 36: 64-73.

*) Brantjes, N.B.M., 1973. Sphingophilous flowers, function of their scent. In: Brantjes, N.B.M., Linskens, H.F. (eds.): Pollination and dispersal. Publ. Dept. Botany, Nijmegen: 26-46.

*) ----- 1976 a. Senses involved in the visiting of flowers by *Cucullia umbratica* (Noctuidae, Lepidoptera). Ent. Exp. & Appl. 20: 1-7.

*) ----- 1976 b. Riddles around the pollination of *Melandrium album* (Mill) Garcke (Caryophyllaceae) during the oviposition by *Hadena bicruris* (Hufn. (Noctuidae, Lepidoptera), I. Proc. Kon. Ned. Akad. Wet. ser. C 79: 1-12.

*) ----- 1976 c. Riddles around the pollination of *Melandrium album* (Mill) Garcke (Caryophyllaceae) during the oviposition by *Hadena bicruris* Hufn. (Noctuidae, Lepidoptera), II. Proc. Kon. Ned. Akad. Wet. ser.C 79: 125-141.

*) ----- 1976 d. Prevention of superparasitation of *Melandrium* flowers (Caryophyllaceae) by *Hadena* (Lepidoptera). Oecologia 24: 1-6.

Darwin, C., 1877. The fertilisation of orchids by insects. London (2nd ed.).

Gilbert, L.E. 1975. Ecological consequences of a coevolved mutualism between butterflies and plants. In: Gilbert, L.E., Raven, P.H. (ed.): Coevolution of animals and plants. Univ. Texas Press, Austin: 210-240.

Gregory, D.P., 1963. Hawkmoth pollination in the genus *Oenothera*. Aliso 5: 357-384.

Heinsius, H.W., 1890. Bijdrage tot de kennis der bestuiving van inlandse bloemen door insecten. Thesis, Amsterdam.

Kislev, M.E., Kraviz, Z., Lorch, J., 1972. A study of hawkmoth pollination by a palynological analysis of the proboscis. Israel J. Bot. 21: 57-75.

Knoll, F., 1922. Lichtsinn und Blütenbesuch des Falters von *Macroglossum stellatarum*. Abh. Zool.-Bot. Ges. Wien 12: 127-378.

----- 1925. Lichtsinn und Blütenbesuch des Falters von *Deilephila Livornica*. Vergl. Physiol. 2: 329-380.

Lederer, G., 1959. Nahrungssuche und Nahrungsaufnahme der in der Dämmerung und in der Nacht fliegenden Lepidopteren. Entomol. Z. 69: 25-136.

Martin, H., 1964. Zur Nahorientierung der Biene im Duftfeld, zugleich ein Nachweis für die Osmotropotaxis bei Insecten. Z. vergl. Physiol. 48: 481-533.

Schremmer, F., 1941. Sinnesphysiologie und Blumenbesuch des Falters von *Plusia gamma* L. Zool. Jahrb. Syst. 74: 375-435.

Tinbergen, N., 1953. Social behaviour in animals. Methuen & Co. London,

----- 1955. Psychology and ethology as supplementary parts of a science of behaviour. In: Schaffner, B., (ed.): Transact. First Conf. Group Process. Macy Foundation, New York: 75-167.

Vogel, S., 1962. Duftdrüsen im Dienste der Bestäubung.
Abh. Math.-Naturw. Kl. Akad. Wiss. Mainz (10): 599-763.

*) = Deze artikelen maken deel uit van dit proefschrift.

AARD DER SYMBIOSE VAN *HADENA BICRURIS* EN *MELANDRIUM ALBUM*

De aard van de relatie tussen *H. Bicruris* en *M. album* kan worden bepaald door de balans op te maken van de schade en het profijt dat ze voor elkaar vormen (Brantjes 1976 c). Voor de vlinder levert de relatie een dubbel voordeel op: voedsel voor zowel de larf als de adult. Het profijt voor de plant kan in de bestuiving liggen. Per vlinderpaar, met een levensduur van ieder plusminus 10 dagen, kan het aantal bestoven bloemen onder optimale omstandigheden geschat worden op maximaal 500. De schade, vraat van de zich ontwikkelende zaden, die de nakomelingen van een vlinderpaar kunnen aanrichten kan meer dan 500 vruchten bedragen. Beperkingen stelt de rups zich niet bij de vraat, en alle vruchten worden volledig leeggegeten. Voor de plant is de symbiose dus nadelig, zodat *H. bicruris* als zaadpredator van *M. album* beschouwd kan worden.

Om vier redenen is *H. bicruris* als een zeer goed aangepaste zaadpredator te zien. a) De eieren worden meestal op bepaalde plaatsen van de plant afgezet: bij het ovarium in de stamperbloemen (Brantjes 1976 a). Dit kan tevens functioneel zijn als bescherming van het ei tegen predatie en uitdroging. b) Tijdens het obligate drinken vóór de oviositie wordt de bloem bestoven, zodat de uit het ei komende rups zich altijd bij een zich ontwikkelende vrucht bevindt. c) Door bij de oviositie wel onderscheid te maken tussen stamper- en meeldraad-bloemen worden alleen eieren gelegd in bloemen waarin de rups voedsel zal aantreffen. Maar door bij het drinken tegelijkertijd geen onderscheid te maken wordt de tong steeds weer voorzien van nieuw pollen zodat de te infecteren bloemen maximaal bestoven worden (Brantjes 1976 b,c). d) Door onderkenning van voorafgaande infectie van de bloem, wordt superparasitering en daarop volgende kannibalisering voorkomen (Brantjes 1976 d).

Dat *M. album* in de natuur toch zaad produceert ondanks het optreden van een effectieve predator kan twee oorzaken hebben:
Ten eerste vinden rupsen nadat ze een vrucht hebben leeggegeten niet altijd alle zich ontwikkelende vruchten aan de plant.
Ten tweede drinken in het veld nog andere vlindersoorten waarvan de rupsen het zaad niet eten uit de bloemen en bestuiven deze ook. In het gebied rond Nijmegen behoort de in dit proefschrift besproken *Cucullia umbratica* (Brantjes 1976 a) daarbij tot de belangrijkste.

REFERENTIES

*) Brantjes, N.B.M., 1976 a. Senses involved in the visiting of flowers by *Cucullia umbratica* (Noctuidae, Lepidoptera). Ent. exp. & appl. 20: 1-7.

*) ----- 1976 b. Riddles around the pollination of *Melandrium album* (Mill). Garcke (Caryophyllaceae) during the oviposition by *Hadena bicruris* Hufn. (Noctuidae, Lepidoptera), I. Proc. Kon. Ned. Akad. Wet. ser. C 79: 1-12.

*) ----- 1976 c. Riddles around the pollination of *Melandrium album* (Mill) Garcke (Caryophyllaceae) during the oviposition by *Hadena bicruris* Hufn. (Noctuidae, Lepidoptera), II. Proc. Kon. Ned. Akad. Wet. ser. C 79: 125-141.

*) ----- 1976 d. Prevention of superparasitation of *Melandrium* flowers (Caryophyllaceae) by *Hadena* (Lepidoptera). Oecologia 24: 1-6.

*) = Deze artikelen maken deel uit van dit proefschrift.

SUMMARY

This thesis presents experimental results dealing with the functioning of flower scent for two groups of night-flying moths (Noctuidae, Sphingidae) at their flower visits.

The behaviour at flower visits and the pollinating process of several Sphingidae (*Manduca sexta, Deilephila elpenor* and *Macroglossum stellatarum*) are described. To the species studied here, flower scent acts as sign-stimulus for the start of the feeding behaviour. A special seeking flight and a change in the reaction to bright and to coloured objects are characteristic of this behaviour. Although the Sphingidae perceive many flower scents and chemicals, the moths react to only a few scents. *D. elpenor* is able to use scent-gradients in locating flowers, but the use of vision alone is also possible.

In contrast to the Sphingidae, which hover, the Noctuidae, *Cucullia umbratica* and *Hadena bicruris*, which are two of the most important local pollinators of *Melandrium album*, always land on the flowers. Several phases of the flower visiting behaviour and the function of the flower scent are described: Activation of the feeding-behaviour, orientation towards the flowers, landing, insertion of the proboscis and for *H. bicruris* females, the egg deposition after drinking. From experiments with *C. umbratica*, it is concluded that the senses for perception of the relative humidity of the air are located on its antennae. This moth is able to locate flowers by smell only, but visual orientation seems to be more predominant. Legs and antennae possess contact chemoreceptors, sensitive to sucrose and to chemicals present on the surface of *M . album* flowers. *C. umbratica* responds after contact of the legs with petal lobes of *M. album* by extension of the proboscis. Inserting behaviour follows only after contact of the proboscis tip with the ligula. This moth appears to be a specialised flower visitor.

Larvae of *H. bicruris* are seed-predators on *M. album*. The adults use the flowers not only to feed on the nectar but also the oviposit. For oviposition, which takes place exclusively into pistillate flowers, an extendible ovipositor is inserted into the flower tube. The egg is mostly attached to the ovary of the flower. Scent provokes the moth to perform a characteristic "seeking flight". Apart from the visual means, *H. bicruris* is also able to locate flowers by the smell alone. In the first instance, the moth is attracted and then decides by the smell whether or not to land. Tests show that the number of landings on objects is proportional to the

intensity of the scent. Extracts of staminate as well as of pistillate flowers receive the same number of visits and, therefore, apparently have an equal intensity of odour. Neither calyx, stigma, ovary or the amount of nectar in the flowers are significant for distinguishing between pistillate and staminate flowers by the ovipositing moth. However, the petals, the ligula as well as the lobe, are important. Selection of the oviposition site may be determined by the smell alone. There exists a quantitative relation between the number of petals and the probability to receive eggs. The feeding behaviour and the oviposition act is always preceded by drinking from the flower, which becomes pollinated in the process. When the drinking is prevented by previous satiation of the moth, the selectivity with regard to oviposition site is lost. The perception of the flower type takes place in the time interval between the finding of a flower and the insertion of the proboscis. Associations, formed during drinking, influence the selection of oviposition site. However, the inborn preference to oviposit into *M. album* flowers is stronger than known associations with other flower species. Nectar consumption is necessary for a steady egg production. One moth consumes about 23 ul. each night. To obtain this quantity, approximately 100 flowers have to be visited. A pair of moths will pollinate up to a maximum of 500 pistillate flowers, during their life time. The progeny of one pair of moths can destruct more than 500 capsules. Therefore, *H. bicruris* is not a mutualistic symbiont but a real predator of *M. album*. Statistical analysis of observational data obtained in the Botanical Garden of the University, Nijmegen, showed that each night most eggs are deposited on certain plants. Second-day flowers receive less eggs than first-day flowers. Flowers containing an egg have a lowered probability of receiving a second one. They have a "mark", which functions only one night. This prevention of super-parasitism, unique for Lepidoptera, is of survival value for the moth species.

CURRICULUM VITAE

Nicolaas Bernardus Maria Brantjes werd op 4 november 1946 geboren te Barneveld. Daar werd ook de lagere school doorlopen. Vanaf september 1959 werd middelbaar onderwijs gevolgd aan het Constantijn College te Amersfoort. In juni 1964 werd het HBS-B diploma behaald. In september dat jaar begon de studie aan de Faculteit Wiskunde en Natuurwetenschappen van de Katholieke Universiteit te Nijmegen. In januarie 1969 werd het candidaatsexamen biologie (letter K) afgelegd. Het doktoraalexamen biologie werd afgelegd in oktober 1971. Tijdens de bewerking van het hoofdvak Geobotanie (Hoofd: Prof Dr. V. Westhoff) onder leiding van Dr. E. van der Maarel werd een stage gelopen op het Rijksinstituut voor Natuurbeheer (R.I.N.) onder leiding van Drs. P. Schoevers. De bijvakken waren Zoologie (Hoofd: Dr. H.C.J. Oomen) onder leiding van Drs. A.M.J. Bouwhuis en Hydrobiologie (Prof. Dr. H.F. Linskens) alsmede Capita uit de Wiskunde (Prof. Drs. J.J. de Jong). In 1972 werd de onderwijsbevoegdheid verkregen. In hetzelfde jaar werd les gegeven aan het Liemers College te Zevenaar. Vanaf november 1971 volgde een aanstelling als wetenschappelijk medewerker aan het Botanisch Laboratorium te Nijmegen (Hoofd: Prof. Dr. H.F. Linskens).

AANTEKENINGEN

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PLATE 1

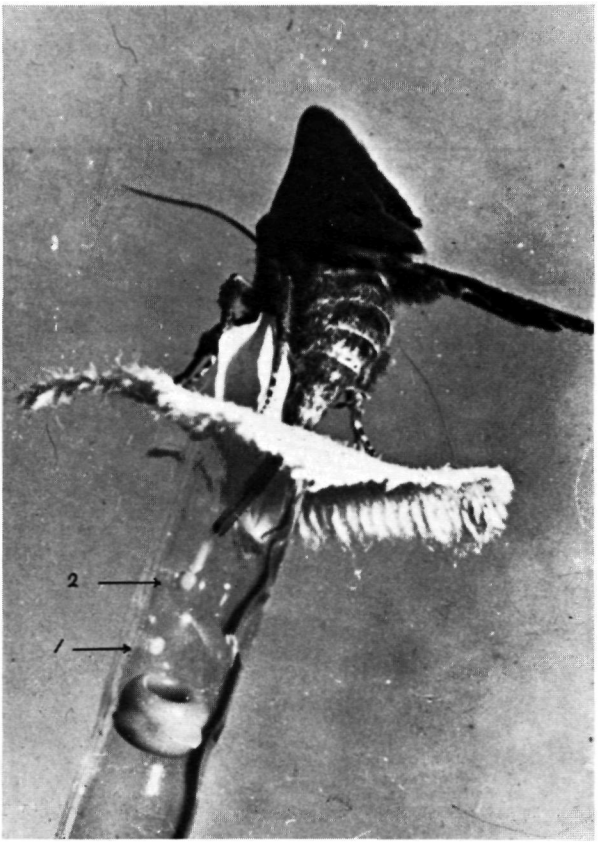

Fig. 1. Oviposition in a pipet flower. *H. bicruris* at the moment the egg (arrow 2) has left the ovipositor. An egg laid by another moth is visable (arrow 1) above the bead, which lays on a constriction in the glass tube. In the opening of the cloth is a petal of *M. album*.

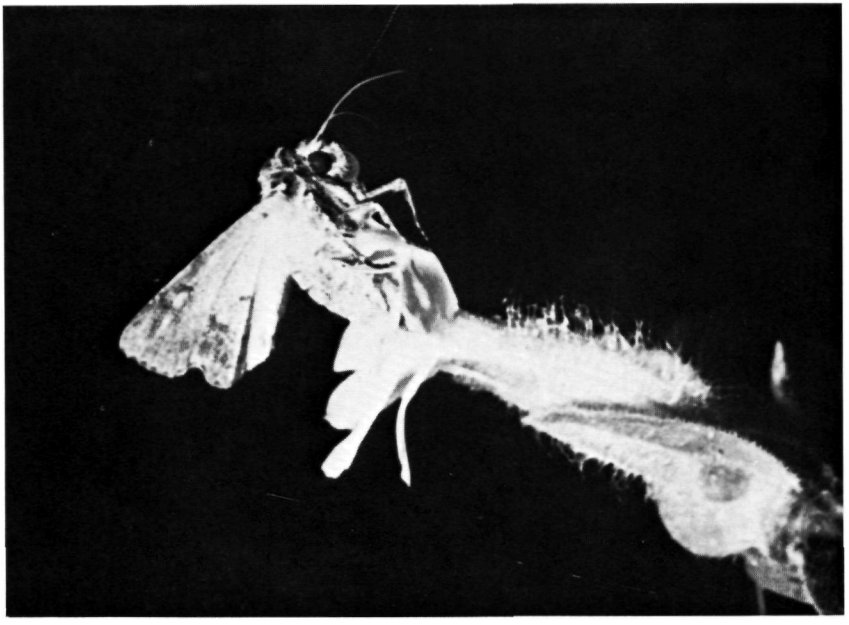

Fig. 2. Oviposition in a pistillate flower. *H. bicruris* forces its abdomen into a flower of *M. album*.

STELLINGEN

I

Het bloembezoekgedrag vormt potentieel een geschikt uitgangspunt bij de bestrijding van schadelijke muggen. Dit gedrag verdient daarom beter onderzocht te worden.

II

De muggen en de kleine nachtvlinders moeten tesamen als een nieuwe bloembezoekers-klasse worden beschouwd.

III

Het schadelijke effect van de temperatuurverhoging door electriciteitscentrales is tot 30°C geringer dan de mechanische schade en de schade door chlorering van het koelwater. Daaróm, en omdat het effect van beide laatstgenoemde schade-oorzaken evenredig is met het gebruikte volume van het koelwater, en deze omgekeerd evenredig is met de toegepaste temperatuurverhoging, is de signaleerbare trend bij de overheid om de grenswaarde voor temperatuur van oppervlaktewater van 30°C terug te brengen naar 25°C een gevaar voor het milieu.

IV

Omdat de door de mens geïntroduceerde honingbij, *Apis mellifica* L., een voedselconcurrent is voor de inheemse bijensoorten zou de honingbij uit de buurt van natuurgebieden moeten worden geweerd.

V

Het "Besluit integrale tandheelkundige hulp ziekenfondsverzekering" (het zogenaamde 13-jarigenplan) legt de formele basis voor het selecteren van een bevoorrechte groep, die medische behandeling van betere kwaliteit ontvangt.

VI

Het kropsecreet van duiven bevat naast voedingsstoffen ook immuunglobulines, die door jonge duiven worden opgenomen. Daarom kan de duif als volwaardig zoogdier worden beschouwd. Dit is een extra argument om de term "Mammalia" niet te gebruiken om de behaarde vertebraten aan te duiden.

N.M.B. Brantjes

Nijmegen, 23 september 1976